

Rensch's rule and sexual dimorphism in salamanders: patterns and potential processes

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Abstract

Body size is influenced by the interaction of multiple forces, whose effects can determine the occurrence of sexual size dimorphism (SSD). Rensch's rule is the increase of SSD with body size in taxa where males are the largest sex, and the opposite pattern in female-biased SSD taxa. This pattern was detected in many animal groups, but contrasting results were also highlighted. This study evaluated the existence of Rensch's patterns for body size and for the number of caudal vertebrae in salamandrid caudate amphibians. Furthermore, we tested the support of alternative hypotheses on processes that may determine allometric patterns: sexual selection, fecundity selection and constraining selection by performing separate analyses on species with male- and female-biased SSD. We used the literature and original data to gather information on body size and number of caudal vertebrae in 52 species of salamandrids over four continents. We then tested the support of the three hypotheses using a phylogenetic approach. Rensch's rule was valid for body size in salamanders only for species with male-biased dimorphism. No allometric relationships were detected by analyses on all the species, or by analyses on female-biased SSD species. Analyses performed on the number of caudal vertebrae showed no significant patterns. Our study supports the role of sexual selection in promoting positive allometry for body size in male-biased SSD species, whereas the alternative hypotheses were not supported by our data. These results highlight the importance of distinguishing male- and female-biased species as different evolutionary pressures and constraints may be at the basis of evolution of SSD in these groups.

Introduction

Rensch's rule is an allometric rule stating that, in groups of related species, sexual size dimorphism (SSD) increases with body size when the males are the largest specimens and decreases with increasing body size when the females are the largest specimens (e.g. Rensch, 1960). This is equivalent to stating that the body size of males varies more over evolutionary time than the body size of females, irrespective of which sex is larger (Blanckenhorn *et al.*, 2007). Rensch's rule has been shown to be valid for various animal groups, including domestic mammals (Polak & Frynta, 2009), birds (Székely, Freckleton & Reynolds, 2004; Dale *et al.*, 2007), reptiles (Frýdlová & Frynta, 2010; Ceballos *et al.*, 2013), anurans (Liao & Chen, 2012; Zhang & Lu, 2013) and insects (Fairbairn, 1994; Blanckenhorn *et al.*, 2007).

Despite the widespread occurrence of Rensch's rule, several studies pointed out the absence of Rensch's rule in some taxa

(Abouheif & Fairbairn, 1997; Fairbairn, 1997; Webb & Freckleton, 2007; Serrano-Meneses *et al.*, 2008; Liao *et al.*, 2013). The validity of the rule has then been questioned, particularly for species with female-biased SSD (Webb & Freckleton, 2007), suggesting the importance of considering male- and female-biased species separately, or to take into account their relative proportions in studied taxa, to understand the underlying mechanisms (see also Abouheif & Fairbairn, 1997). In amphibians, Rensch's rule has been ruled out in a group of anurans as no significant allometric relationship was found between the mean size of males and females (Liao *et al.*, 2013). An opposite pattern, the inverse of Rensch's rule, was found in several female-biased SSD amphibian species (Ivanović *et al.*, 2008; Liao & Chen, 2012). The overall pattern thus remains unclear and needs to be tested in groups composed of male- and female-biased SSD species.

Several hypotheses have been proposed to explain the occurrence of Rensch's patterns. For a particular species, SSD

is determined by the balance between sexual selection, fecundity selection and natural selection. Sexual selection influences traits that enhances breeding success in interactions with sexual partners or competitors and often favours larger males; fecundity selection influences traits enhancing reproductive output (e.g. clutch size) and generally favours larger females, while natural selection (also called survival selection) acts on traits enhancing the probability of survival and may favour larger males or females depending upon the ecological context (Shine, 2000; Blanckenhorn *et al.*, 2007; Dale *et al.*, 2007; Frýdlová & Frynta, 2010; Liao & Chen, 2012; Ceballos *et al.*, 2013). The most frequent explanations are based upon sexual selection: these hypotheses propose that, if sexual selection acts more strongly on one sex, then it would produce a correlated and weaker evolutionary change in the other sex. So, in taxa where sexual selection is stronger on males, positive allometry is expected (and vice versa if females are subjected to strong sexual selection) (Dale *et al.*, 2007). Second, the fecundity selection hypothesis predicts patterns following the inverse of Rensch's rule (Liao & Chen, 2012; Ceballos *et al.*, 2013; Zhang & Lu, 2013). Female size influences reproductive output and is strongly affected by fecundity selection. According to the fecundity selection hypothesis, SSD should increase with the species average body size in female-biased SSD species, or SSD should decrease with body size in male-biased SSD species (Ceballos *et al.*, 2013). Third, the constraining selection hypothesis proposes that selective forces constrain the body size of females more than the body size of males, and this may cause a higher variation of the body size of males along evolution (i.e. Rensch's pattern) in all the species, with both male- and female-biased SSD (Frýdlová & Frynta, 2010). Finally, phylogenetic constraints and inertia may explain a considerable portion of sexual dimorphism. Phylogenetic history should therefore be taken into account in sexual dimorphism analyses to avoid the risk of overstating the importance of selective forces (Cheverud, Dow & Leutenegger, 1986).

Furthermore, analyses of Rensch's rule usually focus upon body size or body size proxies. However, different parts of the body may be subjected to different selective forces. For example, in several caudate amphibians, the size of the tail is strongly related to the balance between sexual and natural selection (Ficetola *et al.*, 2013), while body size may be more strongly related to fecundity selection (Ficetola *et al.*, 2010). Body parts subjected to different selective forces might therefore show different patterns of sexual dimorphism and allometry.

In this study, we analysed sexual dimorphism in salamandrid caudate amphibians to assess the existence of allometric patterns and to evaluate the support of Rensch's rule or inverse Rensch's rule and thus to identify potential selective pressures, such as sexual, fecundity and constraining selection. Specifically, we developed and tested three *a priori* predictions, derived by these hypotheses. (1) If sexual selection is the main driver of allometric variation between sexes, we predict that body size of caudates would follow Rensch's rule, and this would be particularly strong for male-biased dimorphic species, as males are under stronger

intra-sexual competition and male-biased dimorphic species are more subjected to strong sexual selection (e.g. females select for males with specific features, or the males with specific features win intra-sexual competition). (2) If allometric variation is mostly caused by fecundity selection, we predict an inverse Rensch's pattern, especially for female-biased dimorphic species. (3) If allometric variation is caused by the constraints that natural and fecundity selection pose to the body size of females (Frýdlová & Frynta, 2010), we expect an overall Rensch's pattern, irrespective of whether or not species are subjected to strong sexual selection. Finally, if allometric variation of sexual dimorphism is detectable in body traits other than body size, we expect a similar or even stronger pattern for the variation of the number of caudal vertebrae because sexual selection is a major driver of sexual dimorphism for the number of caudal vertebrae (Ficetola *et al.*, 2013).

Materials and method

Data collection

We considered two morphological parameters: snout-vent length (SVL) and number of caudal vertebrae. SVL, measured from the tip of the snout to the posterior edge of the cloaca, is the standard measure of body size in caudate amphibians (Heyer *et al.*, 1994). For tail, we used the number of caudal vertebrae because it is a more objective measure than tail length, it reduces approximation errors, it strongly corresponds to the evolution of tail morphology, it shows sexual dimorphism in several species and it is affected by sexual selection (Ficetola *et al.*, 2013). Furthermore, caudates show tail regeneration, and regeneration may be unnoticeable in analyses of tail length, while regenerated tails can be easily identified and removed from the analysis of caudal vertebrae.

We used the literature and original data to collect information on average SVL and average number of caudal vertebrae for males and females of urodelan amphibians belonging to the Salamandridae family (Salamandrids; Tables 1 and 2; Supporting Information Appendix S1). Salamandridae is the second largest family of urodeles by species number; they comprise about 103 species from North America, Europe, North Africa and Asia, and are the most frequent urodeles in Eurasia (AmphibiaWeb, 2014); we calculated average SVL for 52 species of salamandrids (calculation based upon measures carried out on 21 916 individuals) and average number of caudal vertebrae for 25 species (calculation based upon measures carried out on 1259 individuals). While some authors tested for Rensch's rule between populations (see, e.g. Liao & Chen, 2012), we chose to run the tests at the species level (and thus calculate a single average value for every species) to fit the original definition of Rensch's rule, which refers to allometric relationship among related species. To confirm this approach, we evaluated whether the variability for sexual dimorphism was greater at the inter-specific than at the intra-specific level. We calculated the variance of the sexual dimorphism index (SDI) across species (σ^2_{SDI}) and compared to the average

Table 1 Summary of snout-vent length (SVL, in millimetres) of males and females of salamandrid species

Species	Males			Females		
	<i>n</i>	Range	Mean	<i>n</i>	Range	Mean
<i>Calotriton arnoldi</i>	3	56.3–58.6	57.73	2	57.0–58.9	57.99
<i>Calotriton asper</i>	422	46.9–72.9	61.22	332	47.1–78.1	62.25
<i>Chioglossa lusitanica</i>	94	42.1–49.2	45.18	90	45.9–50.8	46.65
<i>Cynops ensicauda</i>	23	54.0–58.5	55.50	30	58.2–65.4	61.27
<i>Cynops fudigensis</i>	4	43.3–45.7	45.00	6	45.3–52.4	48.75
<i>Cynops pyrrhogaster</i>	346	49.0–57.3	53.55	280	55.7–64.9	61.26
<i>Euproctus montanus</i>	24	36.8–55.5	47.50	55	38.0–56.0	45.90
<i>Euproctus platycephalus</i>	80	53.4–70.2	61.80	45	47.8–58.2	53.00
<i>Ichthyosaura alpestris</i>	1849	40.1–52.9	46.49	1755	43.1–59.3	53.62
<i>Laotriton laoensis</i>	154	72.0–110.0	91.00	120	76.0–114.0	101.00
<i>Lissotriton boscai</i>	135	25.1–37.9	33.46	160	27.2–43.7	38.10
<i>Lissotriton helveticus</i>	314	34.9–41.2	38.25	431	38.8–45.0	41.89
<i>Lissotriton italicus</i>	58	33.2–34.6	33.77	55	35.6–37.6	36.70
<i>Lissotriton montandoni</i>	156	41.1–45.2	43.15	184	47.4–50.9	49.15
<i>Lissotriton vulgaris</i>	683	34.7–43.7	40.15	334	35.3–44.6	40.77
<i>Lyciasalamandra fazilae</i>	42	56.0–71.0	62.26	53	46.0–70.0	60.59
<i>Mertensiella caucasica</i>	19	63.1–73.3	67.53	13	51.2–76.6	63.77
<i>Neureergus crocatus</i>	68	60.3–76.6	65.96	38	67.9–81.6	73.87
<i>Neureergus kaiseri</i>	58	52.4–69.7	61.00	41	55.5–81.4	65.60
<i>Neureergus strauchii</i>	55	69.8–69.6	69.77	26	79.6–78.0	78.87
<i>Notophthalmus perstriatus</i>	261	28.0–38.0	33.50	433	26.0–40.0	33.90
<i>Notophthalmus viridescens</i>	222	40.3–45.1	42.70	210	39.6–43.6	41.60
<i>Ommatotriton ophryticus</i>	367	56.4–78.7	66.61	389	49.9–75.9	57.05
<i>Ommatotriton vittatus</i>	30	47.4–48.3	47.87	30	42.1–44.8	43.53
<i>Pachytriton archospotus</i>	8	75.2–95.5	87.29	13	84.8–109.0	92.09
<i>Pachytriton brevipes</i>	18	77.0–95.4	91.23	7	73.4–83.7	84.29
<i>Pachytriton granulosus</i>	9	59.0–78.9	69.00	11	59.9–81.6	70.90
<i>Pachytriton labiatus</i>	27	74.4–105.3	87.46	24	73.8–106.5	88.44
<i>Paramesotriton chinensis</i>	6	65.0–87.0	78.04	5	78.0–87.0	79.92
<i>Paramesotriton ermizhaoui</i>	8	51.0–86.1	71.72	10	51.4–95.3	70.78
<i>Paramesotriton hongkongensis</i>	408	63.9–69.4	66.88	463	67.1–72.2	70.15
<i>Paramesotriton longliensis</i>	14	63.3–86.3	75.38	13	64.0–93.7	77.20
<i>Paramesotriton yunwuensis</i>	3	97.9–105.2	103.98	5	79.9–88.2	86.55
<i>Pleurodeles nebulosus</i>	8	53.0–68.0	60.58	11	46.0–71.5	59.34
<i>Pleurodeles poireti</i>	16	39.8–49.5	45.49	6	46.0–59.0	50.33
<i>Pleurodeles waltl</i>	158	59.0–114.0	79.70	142	62.0–120.0	83.41
<i>Salamandra algira</i>	5	95.5–106.9	105.18	4	101.0–109.0	107.04
<i>Salamandra atra</i>	81	58.0–75.0	66.50	74	60.0–76.0	68.00
<i>Salamandra corsica</i>	5	84.0–109.0	99.80	6	89.0–120.0	102.67
<i>Salamandra infraimmaculata</i>	149	109.7–137.3	129.43	121	113.8–148.5	134.90
<i>Salamandra lanzai</i>	336	80.2–83.9	82.10	161	80.9–87.3	84.20
<i>Salamandra salamandra</i>	154	102.0–125.0	110.55	243	108.0–127.0	114.43
<i>Salamandrina perspicillata</i>	33	31.5–38.8	33.89	3264	33.6–45.3	38.33
<i>Taricha granulosa</i>	189	51.3–79.0	71.11	122	51.2–74.2	64.05
<i>Triturus carnifex</i>	1410	54.0–80.4	68.18	1384	55.0–84.4	72.59
<i>Triturus cristatus</i>	204	68.4–79.4	72.98	167	71.5–78.0	75.59
<i>Triturus dobrogicus</i>	127	61.9–70.8	66.87	540	65.7–74.4	69.26
<i>Triturus karelinii</i>	132	60.0–82.0	72.45	127	55.0–90.0	75.94
<i>Triturus macedonicus</i>	138	65.8–79.2	73.07	161	65.6–85.4	77.44
<i>Triturus marmoratus</i>	79	71.1–76.4	74.18	106	74.9–85.0	80.29
<i>Triturus pygmaeus</i>	158	47.2–59.4	54.72	176	48.0–61.1	56.64
<i>Tylotriton verrucosus</i>	50	77.2–84.8	81.00	38	90.4–100.0	95.20

These data are extracted from personal measures and from the literature. The complete list of sources is provided in Supporting Information Appendix S1.

Range: SVL, minimum and maximum values for every species; mean: SVL, average value; *n*: number of specimens used to calculate average SVL.

Table 2 Summary of number of caudal vertebrae of males and females of salamandrid species

Species	Males			Females		
	<i>n</i>	Range	Mean	<i>n</i>	Range	Mean
<i>Calotriton asper</i>	12	27–29	28.2	17	26–31	28.6
<i>Euproctus montanus</i>	18	26–32	29.3	17	27–31	29.2
<i>Euproctus platycephalus</i>	21	32–37	34.7	8	31–37	34.0
<i>Ichthyosaura alpestris</i>	100	26–38	31.4	101	27–36	31.5
<i>Lissotriton boscai</i>	11	32–39	34.9	23	29–38	33.9
<i>Lissotriton helveticus</i>	17	30–37	33.1	28	24–34	30.6
<i>Lissotriton italicus</i>	36	31–42	36.1	62	28–39	33.0
<i>Lissotriton montandoni</i>	11	31–37	35.5	10	30–38	33.2
<i>Lissotriton vulgaris</i>	42	26–41	34.0	8	25–37	31.0
<i>Mertensiella caucasica</i>	4	50–59	53.5	6	46–63	52.7
<i>Ommatotriton ophryticus</i>	13	37–46	41.7	12	34–41	38.4
<i>Ommatotriton vittatus</i>	17	31–40	36.2	19	29–34	32.6
<i>Pleurodeles waltl</i>	5	38–41	40.0	6	34–44	41.2
<i>Salamandra atra</i>	11	22–26	24.6	19	24–27	25.4
<i>Salamandra corsica</i>	17	22–27	24.4	22	20–26	24.1
<i>Salamandra infraimmaculata</i>	7	29–31	28.2	3	30–31	29.7
<i>Salamandra lanzai</i>	26	22–26	24.0	20	21–26	23.6
<i>Salamandra salamandra</i>	108	22–31	26.5	96	21–29	26.3
<i>Salamandrina perspicillata</i>	14	34–43	37.6	11	31–45	38.5
<i>Triturus carnifex</i>	61	31–44	36.4	66	33–42	36.6
<i>Triturus cristatus</i>	23	29–39	33.4	19	31–39	35.0
<i>Triturus dobrogicus</i>	13	33–42	36.5	4	32–40	35.5
<i>Triturus karelinii</i>	31	32–44	37.0	31	30–41	36.0
<i>Triturus marmoratus</i>	6	34–44	38.2	17	30–42	36.9
<i>Triturus pygmaeus</i>	5	31–38	35.2	5	28–38	34.6

All data are from Lanza *et al.* (2009) (see also Ficetola *et al.*, 2013).

Range: number of caudal vertebrae, minimum and maximum values for every species; mean: number of caudal vertebrae, average value; *n*: number of specimens used to calculate average values.

variance among populations within species. SDI was calculated on natural-log transformed SVL data, as proposed by Lovich & Gibbons (1992):

$$SDI = [(Larger\ sex / Smaller\ sex) - 1]$$

For both SVL and caudal vertebrae, we tried to obtain data from a consistent number of individuals, focusing upon studies with broad coverage, which gathered data on multiple populations per each species and also on studies summarizing the results of several previously published studies (e.g. Malmgren, 2001). Anyway, with the aim to include as many species as possible and to represent the wide variability of the Salamandridae family, we also included 12 species for which the average SVLs were calculated on less than 10 specimens: recently described species (*Calotriton arnoldi*, *Cynops fudigensis*, *Pachytriton archospotus*, *Pachytriton granulosus*, *Paramesotriton ermizhaoui*, *Paramesotriton yunwuensis*) and species generically characterized by few data (*Pachytriton brevipes*, *Paramesotriton chinensis*, *Pleurodeles nebulosus*, *Pleurodeles poireti*, *Salamandra algira*, *Salamandra corsica*). Data on the average number of caudal vertebrae were obtained from Lanza, Arntzen & Gentile (2009) (see also Ficetola *et al.*, 2013).

Several SVL measures were obtained from preserved specimens, and preserved specimens are often subjected to shrinkage of tissues (Verrel, 1985). When possible, we used data from studies using live specimens and discarded those from collections. When needed, SVL from preserved specimens was corrected according to the known values of tissue shrinkage. Studies comparing live and conserved specimens estimated an average shrinkage of 4.4% (SE = 1.2%) (Verrel, 1985; Lanza *et al.*, 2007). Data were corrected as follows:

$$SVL_{corrected} = SVL_{preserved} + SVL_{preserved} \times 0.044$$

Although shrinkage may be different depending upon preservation liquid and concentration, the limited variation between studies suggests that this approximation may help to avoid down-biased estimation of SVL averages.

Statistical analyses

We used phylogenetic major axis regression (PRMA) to test for Rensch's rule in SVL and in the number of caudal vertebrae. In analyses of sexual dimorphism, both *x* and *y* variables are estimated with errors, and there was no *a priori* reason to

use one or the other as the dependent variable; therefore, major axis regression (which accounts for error in the independent variable) should be used instead of least square regression (Sokal & Rohlf, 1995). Phylogenetic reduced major axis regression allows accounting for the shared evolutionary history of species which otherwise may determine false correlations between traits (Revell, 2010). Phylogeny was integrated into PRMA using the phylogenetic trees by Pyron & Wiens (2011) to represent relationships among studied species. The tree was then pruned to include only the species used for the analyses, and recently described species were added according to the respective phylogenetic position following references in Supporting Information Appendix S1; these operations were performed using the package *ape* (Paradis, Claude & Strimmer, 2004).

We interpreted the results of PRMA as follows:

- (1) If the slope of the regression of SVL and number of caudal vertebrae of males on females is significantly greater than 1, then SSD evolves following Rensch's rule.
- (2) If the slope is significantly less than 1, then SSD evolves opposite to Rensch's rule.
- (3) If the slope is not significantly different from 1, SSD evolves in overall isometry with body size.

For each trait (SVL and caudal vertebrae), we repeated PRMA three times. First, we analysed all the species. Subsequently, to test the support of hypothesis on the evolution of SSD (see the Introduction section), the analysis was repeated using only male-biased sexual dimorphic species (12 species) and using only female-biased sexual dimorphic species (40 species). We also repeated analyses removing from the database all the species with small sample size to test the robustness of our results. PRMA was performed using package *phytools* (Revell, 2012).

To test for the significance of the phylogenetic signal in SSD, we built a phylogenetic generalized least squares (PGLS) model relating male SVL to female SVL. We used maximum likelihood to estimate the parameter lambda (Pagel, 1999). If lambda is significantly greater than zero, there is evidence of significant phylogenetic signal in the relationship. PGLS was performed using package *caper* (Freckleton, Harvey & Pagel, 2002). Data on SVL were log-transformed to improve normality; all analyses were performed under the R 2.14.1 statistical environment (Copyright © 2011 The R Foundation for Statistical Computing).

Results

We obtained data on SVL for 52 species from the Salamandridae family (Table 1) (genera *Calotriton*, *Chioglossa*, *Cynops*, *Euproctus*, *Ichthyosaura*, *Laotriton*, *Lissotriton*, *Lyciasalamandra*, *Mertensiella*, *Neurergus*, *Notophthalmus*, *Ommatotriton*, *Pachytriton*, *Paramesotriton*, *Pleurodeles*, *Salamandra*, *Salamandrina*, *Triturus*, *Tylostotriton*), and data on caudal vertebrae for 25 species (Table 2). For SVL, the inter-specific variability of sexual dimorphism was two orders of magnitude greater than the intra-specific variability (inter-specific level: $\sigma^2_{SDI} = 0.004$; intra-specific

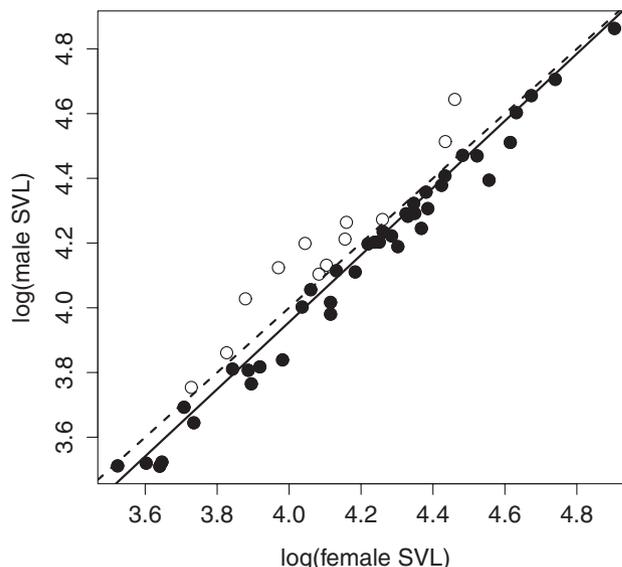


Figure 1 Relationship between male body size and female body size (log of average snout-vent length, measured in millimetres) in 52 salamandrid species. The dashed line indicates isometry and the solid line represents the PRMA model fitted to the data. Black circles are species in which females are the largest sex, whereas white circles are species in which males are the largest sex.

level: $\sigma^2_{SDI} = 0.00005$). Therefore, intra-specific variability was limited and overwhelmed by the inter-specific one.

PRMA performed on SVL using all the species showed no significant allometric relationship between females and males ($P = 0.450$). Similarly, we did not observe any significant allometric relationship if female-biased dimorphic species are analysed separately ($P = 0.418$) (Figs 1 and 2; Table 3). The analyses using only well-sampled species gave similar results (Table 3). If only male-biased dimorphic species are considered, we detected a significant positive allometry (slope = 1.226, $P = 0.028$) (Fig. 3; Table 3). The allometry remained clearly detectable and significant when the analysis was repeated using only well-sampled species ($n > 10$) (Table 3). The maximum likelihood estimate of lambda was not significantly higher than zero ($P = 0.470$), suggesting a weak phylogenetic signal.

PRMA performed on caudal vertebrae showed no significant allometric relationship between females and males (Fig. 4; Table 3). No significant relationship was detected either in species with male-biased dimorphism or in species with female-biased dimorphism, even if they were analysed separately (Table 3).

Discussion

Rensch's rule is an allometric pattern of sexual dimorphism that has been detected in multiple taxa. Its causes are various and widely debated, and studies performed on a range of taxa

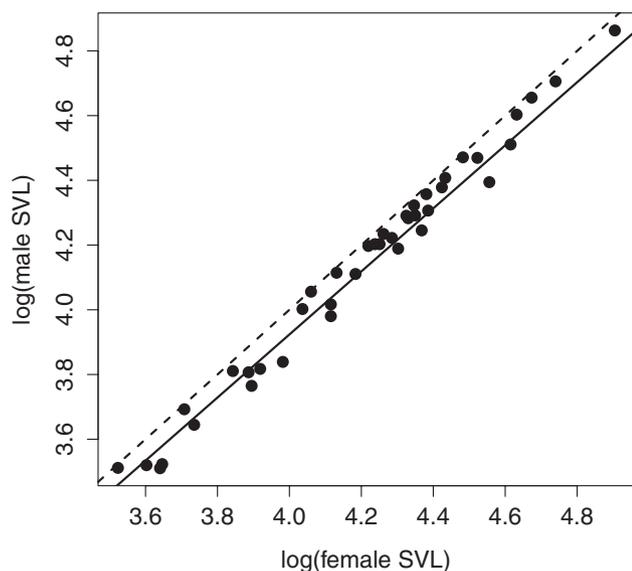


Figure 2 Relationship between male body size and female body size (log of average snout-vent length, measured in millimetres) in 40 species in which females are the largest sex. The dashed line indicates isometry and the solid line represents the PRMA model fitted to the data.

provided support for different underlying processes, such as sexual, fecundity and constraining selection. At least three potential patterns of SSD allometry can be expected, and each one might reveal a different role for these selective forces. Our analysis shows that Rensch's rule is valid for body size in salamandrid caudate amphibians for species with male-biased SSD only and supports the role of sexual selection in promoting Rensch's patterns.

For species with male-biased SSD, we detected a positive allometric relationship between the SVL of females and males, indicating that SSD is stronger and more male-biased in the largest species (Fig. 2). Conversely, neither Rensch's rule nor its inverse was valid for female-biased dimorphic species. In salamandrids, males are generally in competition for mates and may exhibit conspicuous secondary sexual characters and complex courtship displays (Griffiths, 1995; Wiens, Sparreboom & Arntzen, 2011). Sexual selection is the hypothesis most frequently proposed to explain allometric patterns following Rensch's rule (Wiklund & Forsberg, 1991; Fairbairn, 1997; Székely *et al.*, 2004; Dale *et al.*, 2007; Polak & Frynta, 2009). Our data support sexual selection as the main driver of the observed allometry, as sexual selection is expected to be strongest in species with male-biased sexual dimorphism. Actually, some of the species with evident male-biased SSD (e.g. *Ommatotriton ophryticus*, *Ommatotriton vittatus*) are among the salamandrids in which males show the most complex courtship behaviour and exhibit the most conspicuous sexual ornamentation (Wiens *et al.*, 2011; Ficetola *et al.*, 2013).

Our data do not support the expectations of the constraining selection hypothesis. This hypothesis proposes that natural and

fecundity selection pose stronger constraints on females. This would lead to a similar allometric pattern for all the species, irrespective of the direction of their dimorphism. In our study case, the slope of the relationship between male and female SVL was significantly different among male- and female-biased SSD species (PRMA: $P = 0.016$), and no allometric relationship was detected if all species are analysed together. The importance of constraining selection has been advocated mostly for reptiles (Bonnet *et al.*, 2011; Ceballos *et al.*, 2013), in taxa for which differences in breeding strategies are not considered to be particularly strong. Finally, our results are in disagreement with the fecundity selection hypothesis. Fecundity selection has been proposed as the major determinant of SSD allometry in several studies, mainly in anuran amphibians (Liao & Chen, 2012; Zhang & Lu, 2013) and fish (Herczeg, Gonda & Merila, 2010) and is expected to determine an inverse Rensch's pattern in female-biased dimorphic species.

We did not detect significant allometry when analysing the number of caudal vertebrae. This may be caused by several factors. First, only five species (*O. ophryticus*, *O. vittatus*, *Lissotriton vulgaris*, *Lissotriton italicus* and *Lissotriton helveticus*), within the 25 species that we used in our analyses, are characterized by strong sexual dimorphism for the number of tail vertebrae (Ficetola *et al.*, 2013). Second, in caudate species, sexual selection targets several body parts other than the tail (e.g. dorsal crests, limbs and cloaca) (Malmgren & Thollesson, 1999; Wiens *et al.*, 2011) and it is possible that some of these characters show a stronger variation in response to sexual selection, compared to the number of caudal vertebrae. Finally, tail morphology is also correlated with the lifestyle and habitat (Gvoždík & Damme, 2006). Even if several studies identified the important role of sexual selection, natural selection is an important driver of the evolution of tail morphology and therefore could obscure patterns of sexual selection.

SSD is determined by the balance and interaction of multiple selective forces, and evaluating their role over evolutionary scales may be complex. Analysing patterns of allometry in SSD can provide insights on the importance of different selective forces for the evolution of SSD. Several approaches can provide a more explicit and clear identification of selective forces determining sexual dimorphism. First, information from multiple sources, ranging from behavioural ecology to molecular phylogeny, can be integrated to make more complete the results of analyses (e.g. Wiens *et al.*, 2011). Furthermore, hypotheses on the processes determining sexual dimorphism can be used to produce distinct *a priori* predictions, which, in turn, can be explicitly tested. This hypothetical-deductive approach can allow a more objective identification of ongoing processes and therefore greatly help to unravel the causes of sexual dimorphism patterns (Dale *et al.*, 2007; Webb & Freckleton, 2007; McIntire & Fajardo, 2009).

Our study analysed only a limited number of species, particularly for male-biased SSD species. Nevertheless, the study species cover reasonably well the diversity of the Salamandridae family, as we considered about 89% of the European species (Sillero *et al.*, 2014), about 55% of the total Salamandridae family (AmphibiaWeb, 2014), and we

Table 3 Results of phylogenetic major axis regression relating morphological parameters of males to parameters of females

Parameter	Analysis	<i>n</i>	Slope	<i>R</i> ²	<i>t</i>	d.f.	<i>P</i>
SVL	All species	52	1.036	0.889	0.763	36.6	0.450
	Female-biased species	40	0.974	0.962	0.820	27.6	0.418
	Male-biased species (all species)	12	1.226	0.939	2.462	8.8	0.028
	All species (species with <i>n</i> > 10 only)	40	0.967	0.920	0.731	28.0	0.470
	Female-biased species (species with <i>n</i> > 10 only)	32	0.956	0.952	1.109	22.3	0.278
Caudal vertebrae	Male-biased species (species with <i>n</i> > 10 only)	8	1.248	0.967	3.010	6.0	0.023
	All species	25	1.042	0.888	0.601	17.9	0.554
	Female-biased species	8	0.993	0.952	0.073	6.0	0.943
	Male-biased species	17	1.018	0.957	0.337	12.1	0.741

The test evaluates whether the slope of the relationship is significantly different from one for all species, for species with female-biased sexual dimorphism, and for species with male-biased sexual dimorphism.

d.f., degrees of freedom; *n*, number of species; SVL, snout-vent length.

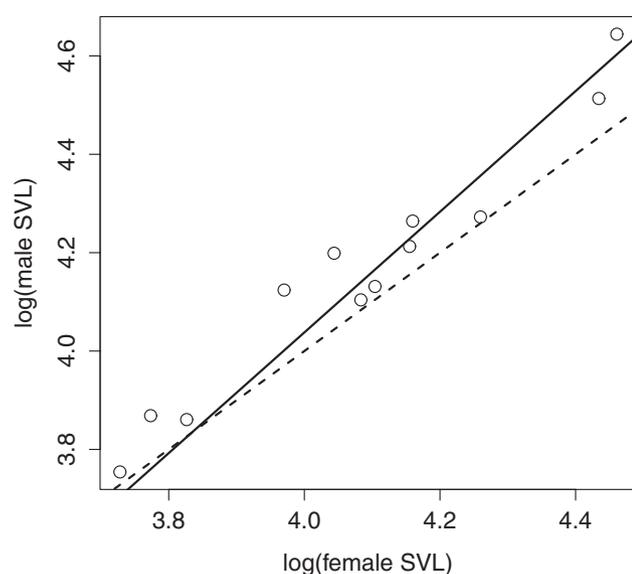


Figure 3 Relationship between male body size and female body size (log of average snout-vent length, measured in millimetres) in 12 species in which males are the largest sex. The dashed line indicates isometry and the solid line represents the PRMA model fitted to the data.

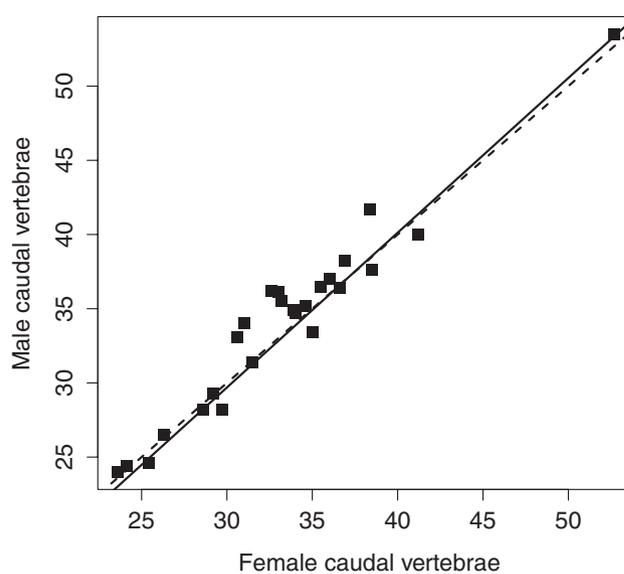


Figure 4 Relationship between the number of caudal vertebrae in males and the number of caudal vertebrae in females. The dashed line indicates isometry and the solid line represents the PRMA model fitted to the data.

included species from four continents. Our analysis was constrained by data availability, especially for caudal vertebrae, but analyses with broader taxonomic and geographic coverage, or encompassing variability at the intra-specific level (e.g. population or subspecies) (Denoël *et al.*, 2009), will certainly help a more complete understanding of the selective forces that are operating.

Separating species with male SSD from species with female SSD during the analyses helps a better understanding of the mechanisms underlying variation in sexual dimorphism and the potential causes of inter-specific variation determining Rensch's rule (Dale *et al.*, 2007). In this view, our results support the evidence that the Rensch's rule criterion about male-biased SSD species (i.e. SSD increases with body size

when the males are the largest specimens) is more generally supported than the criterion about female-biased SSD (i.e. SSD decreases with body size when females are the largest specimens).

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Summary of snout-vent length (SVL, in mm) of males and females of salamandrids species with bibliographic references.