

# Evolution of Sexual Dimorphism in the Number of Tail Vertebrae in Salamanders: Comparing Multiple Hypotheses

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**Abstract** The evolution of sexual dimorphism is an important topic of evolutionary biology, but few studies have investigated the determinants of sexual dimorphism over broad phylogenetic scales. The number of vertebrae is a discrete character influencing multiple traits of individuals, and is particularly suitable to analyze processes determining morphological variation. We evaluated the support of multiple hypotheses concerning evolutionary processes that may cause sexual dimorphism in the number of caudal vertebrae in Urodela (tailed amphibians). We obtained counts of caudal vertebrae from >2,000 individuals representing 27 species of salamanders and newts from Europe and the Near East, and integrated these data with a molecular phylogeny and multiple information on species natural history. Per each species, we estimated sexual dimorphism in caudal vertebrae number. We then used phylogenetic least squares to relate this sexual dimorphism to natural history features (courtship complexity, body size dimorphism, sexual ornamentation, aquatic phenology) representing alternative hypotheses on processes that may explain sexual dimorphism. In 18 % of species, males had significantly more caudal vertebrae than females, while in

no species did females have significantly more caudal vertebrae. Dimorphism was highest in species where males have more complex courtship behaviours, while the support of other candidate mechanisms was weak. In many species, males use the tail during courtship displays, and sexual selection probably favours tails with more vertebrae. Dimorphism for the number of tail vertebrae was unrelated to other forms of dimorphism, such as sexual ornamentation or body size differences. Multiple sexually dimorphic features may evolve independently because of the interplay between sexual selection, fecundity and natural selection.

**Keywords** Sexual selection · Fecundity selection · Newts · Caudata · Behaviour · *Ommatotriton* · *Lissotriton*

## Introduction

Morphological differences between sexes (i.e., sexual dimorphism) are caused by the complex selective forces having different effects on males and females. On the one hand, sexual selection may favour morphological differences that provide advantages during mate selection, such as traits involved in intrasexual dominance and male ornamentation. On the other hand, fecundity selection can determine morphological features improving reproductive output, such as greater size of females in oviparous species, or structures favouring parental care (e.g., mammary gland, pouch). Finally, natural selection favours morphological traits enhancing the organism's survival regardless of its reproductive role. These three forces will act jointly, determining degree and kind of sexual dimorphism (for discussion, see Mayr 1972; Shine 2000; Blanckenhorn 2005; Allen et al. 2011; Wiens et al. 2011). The identification of processes determining sexual dimorphism is an

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important topic for evolutionary biology, yet few studies investigated the evolution of sexual dimorphism over broad phylogenetic scales (Wiens et al. 2011).

In reptiles, fishes and amphibians the number of vertebrae can show strong variation both among and within species. For instance, in several species of fish, populations living at high latitudes have more vertebrae (Jordan's rule), and this has been interpreted as an adaptation to cold climates (McDowall 2008). In caudate amphibians, the number of vertebrae is used as a key morphological character to reconstruct both intraspecific and interspecific phylogeny (Veith 1994; Lanza et al. 2009; Wielstra and Arntzen 2011). The number of vertebrae is a discrete morphological feature that can be measured objectively. It has a strong genetic basis (Itazawa 1959; Shine 2000), is fixed for each individual, being determined early during embryonic development, and can influence multiple traits such as body size, locomotory performance and other behavioural characters (Shine 2000; McDowall 2008). For these reasons, the number of vertebrae is an excellent character to use in analysis of evolutionary processes determining morphological variation. For instance, intra-specific variation in the number of vertebrae helped to identify causes of sexual dimorphism in snakes, and allowed the formulation of hypotheses on relationships between temperature and morphological variation in fish (Shine 2000; McDowall 2008). Nevertheless, very little is known of the evolutionary processes that may determine variation in the number of vertebrae in amphibians.

Sexual dimorphism in the number of tail vertebrae (caudal vertebrae sexual dimorphism: CVSD) has been observed in several taxa of Urodela (newts and salamanders) (Lanza et al. 2009). However, the causes of this dimorphism are unclear. Multiple, non mutually-exclusive hypotheses can be proposed to explain the occurrence of CVSD in Urodela (Table 1), and lead to testable predictions that can be used to evaluate the support for underlying processes. These hypotheses assume different roles for natural, sexual and fecundity selection, as follows (Table 1):

(a) **Body size dimorphism hypothesis.** The interplay between natural, fecundity and sexual selection determines sexual dimorphism in body size. Male-biased size dimorphism is often observed when sexual selection prevails, fecundity selection favours female-biased dimorphism, and allometric relationships between the size of the two sexes may drive dimorphism over broad taxonomic ranges (Rensch's rule) (Shine 2000; Blanckenhorn 2005; Dale et al. 2007; Stephens and Wiens 2009; Frydlova and Frynta 2010; Allen et al. 2011; Ceballos et al. 2012). CVSD may occur just as a by-product of sexual dimorphism of body size, for instance because of genetic

correlations between body size and tail features (e.g., tail length) (Dale et al. 2007). This hypothesis therefore predicts a positive relationship between CVSD and dimorphism for body size.

- (b) **Courtship hypothesis.** In many Urodela, the tail is involved in courtship behaviours, and allows to present visual and chemical stimuli to the female (Griffiths 1995; Wiens et al. 2011). According to this hypothesis, sexual selection would promote CVSD in species with complex courtship behaviours, because tails with more vertebrae may be longer, more visible or more flexible.
- (c) **Sexual ornamentation hypothesis.** In many Urodela sexual selection promoted the evolution of complex sexual ornamentation in males, both for tail and body (e.g., crests) (Griffiths 1995; Wiens et al. 2011). If multiple sexual characters co-evolve, CVSD may be highest in species where males are more ornate for other sexual characters, such as dorsal crests.
- (d) **Aquatic phase hypothesis.** Tail features and the number of vertebrae affect swimming performance (Gvozdik and Van Damme 2006; McDowall 2008), therefore CVSD may be affected by the length of the aquatic phase. If tail features are optimized to improve swimming performance, stabilizing selection would act to maintain a consistent number of vertebrae in more aquatic species (i.e., species spending more time in water during either the larval or the adult life history stages), therefore limiting dimorphism.
- (e) **Aquatic courtship hypothesis.** In several species, tail is involved in the wafting of pheromones toward females, and this behaviour can be particularly important when courtship is aquatic (Griffiths 1995; Zug et al. 2001); this hypothesis predicts higher CVSD in species that have a complex courtship, but only if the courtship is aquatic. This may be detectable as a statistical interaction between complexity of courtship and the occurrence of aquatic courtship.

The aim of this study was to evaluate the potential processes that may explain the occurrence of sexual dimorphism for the number of caudal vertebrae in Urodela. First, we analyzed data on the number and caudal vertebrae of Urodela of the Western Palaearctic, to assess whether species show CVSD. Subsequently, we used an information-theoretic approach to test the support of our five a priori hypotheses that may explain the occurrence of CVSD.

## Methods

We gathered data on the number of caudal vertebrae in species of Urodela of Europe and the Near East (including

**Table 1** Hypotheses proposed to explain sexual dimorphism for caudal vertebrae (CVSD) in Urodela

Hypothesis	Independent variable	Predicted relationship	Selective force(s)
(a) <i>Body-size dimorphism</i> : CVSD is a by-product of sexual dimorphism of body size	Sexual dimorphism of body size (rank scale) (Griffiths 1995; Grossenbacher and Thiesmeier 2003; Lanza et al. 2007; Raffaelli 2007; Ivanovic et al. 2008; Arakelyan et al. 2011)	+	Natural, sexual and fecundity selection
(b) <i>Courtship</i> : CVSD caused by complex courtship behaviours	<i>N</i> of courtship behaviours (Nöllert and Nöllert 1992; Grossenbacher and Thiesmeier 2003; Lanza et al. 2006; Lanza et al. 2007; Wiens et al. 2011)	+	Sexual selection
(c) <i>Ornamentation</i> : CVSD related to other sexual ornamentations	<i>N</i> of crest characters (Wiens et al. 2011)	+	Sexual selection
(d) <i>Aquatic phase</i> : CVSD negatively impacts swimming performance: the <i>absolute value</i> of CVSD is lower in more aquatic species	Two variables: Length of aquatic phase of larvae; length of aquatic phase of adults (Nöllert and Nöllert 1992; Griffiths 1995; Grossenbacher and Thiesmeier 2003; Lanza et al. 2007; Arakelyan et al. 2011)	–	Natural selection
(e) <i>Aquatic courtship</i> : CVSD related to complex courtship, but only if courtship is aquatic	<i>N</i> of courtship behaviours + interaction between <i>N</i> of courtship behaviours and the occurrence of aquatic courtship (Griffiths 1995; Grossenbacher and Thiesmeier 2003; Lanza et al. 2007; Wiens et al. 2011). See Appendix S1	Positive relationship with courtship in aquatic species only	Sexual selection

Turkey, Israel and Western Iran) from the literature (reviewed by Lanza et al. 2009). Lanza et al. (2009) combined original data and a complete review of previously published information on vertebral count in the Urodela of the Western Palaearctic; these data have been mostly obtained through X-ray photographs. We considered only individuals for which data on both sex and collection locality were available; the dataset did not include individuals with regenerated/damaged tail. To allow sufficient statistical power, we considered only species for which at least 10 individuals per sex were available.

Natural history data used to test the five hypotheses were obtained from monographs summarizing the available information on the study species, or from research papers reporting information on multiple populations and wide areas (see Table 1 for references). For body-size sexual dimorphism, species were classified using a rank scale as (1) sexually dimorphic, females larger; (2) no significant dimorphism for body size; (3) dimorphic, males larger. The number of courtship-related behavioural traits of males, and the number of characters of the dorsal crest, were used to quantify complexity of the courtship and sexual ornamentation, respectively (Wiens et al. 2011). Using presence/absence of dorsal crest instead of the number of crest characters yielded identical results (not shown). The length of aquatic phase was quantified using two metrics: as the length of aquatic larval phase (in months), and as the number of months per year spent in water by adults; for terrestrial-only species, length of aquatic phase was set to zero. In some species, populations living in different regions may have different length of aquatic phases, and

we used the midpoint of the minimum and maximum length of aquatic phases.

We used published data on the phylogeny and divergence time of study species, to build a tree representing phylogenetic divergence among species (Appendix S1 in Supplementary Online Material). For Salamandridae we used the time-calibrated phylogeny based on both mitochondrial and nuclear data published by Wiens et al. (2011). For Plethodontidae, we used the time-calibrated mitochondrial phylogeny published by Carranza et al. (2008). Note that the position of plethodontid species considered in this study remained identical in analyses combining mitochondrial and nuclear data (van der Meijden et al. 2009). Divergence among families followed Zhang and Wake (2009).

#### Statistical Analyses

We used Student's *t* tests to evaluate whether the number of caudal vertebrae was significantly different between sexes within species. The number of vertebrae is sometimes different among subspecies (Lanza et al. 2009). For species in which vertebral counts were obtained from individuals of multiple subspecies, we analyzed data using mixed models instead of *t* tests, including subspecies as random factor. We used sequential Bonferroni's correction to take into account the large number of tests performed (Rice 1989). For all species, the assumptions of homogeneity of variance and normality of residuals were not violated.

We used an information-theoretic approach to identify the hypotheses most likely to explain the interspecific

variation for CVSD (McIntire and Fajardo 2009; Symonds and Moussalli 2011). We used Cohen's  $d$  as a measure of the effect size of the difference in mean number of vertebrae between males and females (Hartung et al. 2008). For the majority of study species, males were the sex with more caudal vertebrae (see Results); positive values of Cohen's  $d$  indicate species in which males are the sex with more vertebrae, and vice versa. The Lovich and Gibbons (1992) Sexual Dimorphic Index (SDI) is another frequently used measure of sexual dimorphism. Nevertheless, Cohen's  $d$  was very strongly related to SDI (Pearson's correlation,  $r = 0.96$ ), and all results remain unchanged if the SDI was used instead of Cohen's  $d$  (not shown).

We used phylogenetic generalized least squares (PGLS) to assess the relationship between Cohen's  $d$  and the variables representing hypotheses explaining CVSD (Table 1). PGLS incorporates phylogenetic non-independence among species in the error term, rather than assuming independent error among species, and is therefore particularly suitable for investigation of evolutionary relationships between traits (Martins and Hansen 1997; Freckleton et al. 2002, 2011). First, we built models relating Cohen's  $d$  to the variables representing the five a priori hypotheses (Table 1). We built models considering only one hypothesis (a, b, c or e) at each time; subsequently, we built complex models representing multiple hypotheses (i.e., a + b, a + c, a + e, b + c, c + e). The aquatic phase hypothesis was not included among these candidate models, because it had a different dependent variable (see below). We then calculated the Akaike's Information Criterion corrected for small sample size (AICc) for each model. AICc is a numerical value allowing us to rank competing candidate models in terms of information loss in approximating the (unknowable) truth (Symonds and Moussalli 2011). AICc trades off explanatory power versus number of predictors; parsimonious models explaining more variation have the lowest AICc values and are considered to be the "best" models (Symonds and Moussalli 2011). AICc may select overly complex models; therefore we considered a complex model as a candidate only if it had an AICc less than the AICc of all of its simpler nested models (Richards et al. 2011). Simulations showed that this approach improves the identification of best model (Richards et al. 2011). For each candidate model, we calculated the Akaike's weight  $w$  (AICc weight), representing the probability that a model is the best one, given the data (Lukacs et al. 2007; Symonds and Moussalli 2011). This analysis was repeated three times (considering all species, considering Salamandridae only, and setting Cohen's  $d$  to zero in species where CVSD was non-significant) to confirm the robustness of our results.

In the previous models, CVSD was considered as the dependent variable. However, the aquatic phase hypothesis

predicts no sexual dimorphism for the aquatic species; rather, it predicts that the absolute value of CVSD is lower in species that are more aquatic either during the larval phase, or during the adult phase. To test this hypothesis, we built two models, the first relating the absolute value of CVSD to the average length of aquatic phase of larvae, and the second relating the absolute value of CVSD to the aquatic phase of adults. This was the only hypothesis in which the absolute value of CVSD was the dependent variable: thus, the two models could not be compared with candidate models representing different hypotheses. We therefore compared these two models between them and with the null model (Lukacs et al. 2007). In three species (*Salamandrina perspicillata*, *Salamandra salamandra* and *Salamandra corsica*) only females go to breeding wetlands. The relationship between CVSD and the aquatic phase of adults was re-analyzed, by setting length of aquatic phase = 0 in these three species.

For all models, we also calculated adjusted  $R^2$  ( $R^2_{\text{adj}}$ ) as a measure of the amount of variation explained (Borcard et al. 2011). The length of aquatic phase was square-root transformed prior to analyses to reduce skewness. We performed analyses under the R statistical environment (R Development Core Team 2012) using packages APE 3.0 and CAPER 0.5 for phylogenetic analyses (Paradis et al. 2004; Orme et al. 2012) and COMPUTE.E5 0.2 to estimate effect size (Del Re 2012).

## Results

We obtained data for 2078 individuals corresponding to 27 species of Urodela (Plethodontidae: genus *Hydromantes*, 7 species; Proteidae, genus *Proteus*, 1 species, Salamandridae: genus *Salamandrina*, 1 species; *Salamandra*, 4 species; *Euproctus*, 2 species; *Ichthyosaura*, 1 species; *Lissotriton*, 5 species; *Ommatotriton*, 2 species; *Calotriton*, 1 species; *Triturus*, 3 species—Table 2). The average sample size ( $\pm$ SD) was  $77.0 \pm 66.4$  individuals per species (Table 2). After sequential Bonferroni's correction, we detected significant intersexual differences in caudal vertebrae number for five species: *Lissotriton vulgaris*, *L. italicus*, *L. helveticus*, *Ommatotriton ophryticus* and *O. vittatus*. For all these species, males had tails with significantly more caudal vertebrae (modal difference between males and females: *L. italicus* and *L. helveticus*: 2 vertebrae; *L. vulgaris*, *O. ophryticus* and *O. vittatus*: 3 vertebrae) (Table 2).

### Hypotheses to Explain Dimorphism of Caudal Vertebrae

The model representing the courtship hypothesis showed the lowest AICc value, and was thus considered to be the

**Table 2** Average number of vertebrae in males and females of 27 species of caudate amphibians, and results of *t* tests or mixed models

Species	<i>N</i>	<i>N</i> vertebrae		<i>t</i>	<i>df</i>	<i>P</i>	Cohen's <i>d</i>
		Males	Females				
<i>Triturus cristatus</i>	42	33.43	34.95	−2.271	40	0.029	−0.70
<i>Triturus carnifex</i>	132	36.39	36.55	−0.398	130	0.691	−0.07
<i>Triturus karelinii</i>	58	37.00	35.97	1.320	53	0.192	0.35
<i>Calotriton asper</i>	29	28.18	28.56	−0.869	27	0.393	−0.33
<i>Ommatotriton ophryticus</i> <sup>a</sup>	25	41.69	38.42	3.587	23	<b>0.002</b>	1.44
<i>Ommatotriton vittatus</i> <sup>a</sup>	36	36.18	32.63	5.113	33	<b>&lt;0.001</b>	1.71
<i>Lissotriton vulgaris</i> <sup>a</sup>	184	33.95	30.99	6.856	179	<b>&lt;0.001</b>	1.03
<i>Lissotriton montandoni</i>	21	34.45	33.20	1.317	19	0.204	0.58
<i>Lissotriton italicus</i>	98	36.06	32.95	5.950	96	<b>&lt;0.001</b>	1.26
<i>Lissotriton helveticus</i>	45	33.06	30.61	3.364	43	<b>0.002</b>	1.03
<i>Lissotriton boscai</i>	34	34.91	33.87	1.286	32	0.208	0.47
<i>Ichthyosaura alpestris</i> <sup>a</sup>	201	31.42	31.48	−0.117	196	0.907	−0.02
<i>Euproctus montanus</i>	35	29.33	29.24	0.198	33	0.844	0.07
<i>Euproctus platycephalus</i>	29	34.67	34.00	1.070	27	0.294	0.44
<i>Salamandra salamandra</i> <sup>a</sup>	204	26.48	26.34	0.702	201	0.484	0.10
<i>Salamandra lanzai</i>	46	24.04	23.62	1.069	44	0.291	0.32
<i>Salamandra atra</i> <sup>a</sup>	32	24.58	25.35	−1.573	28	0.127	−0.57
<i>Salamandra corsica</i>	39	24.35	24.05	0.652	37	0.518	0.21
<i>Salamandrina perspicillata</i>	25	37.64	38.45	−0.634	23	0.532	−0.26
<i>Proteus anguinus</i>	22	29.00	28.45	0.582	20	0.545	0.24
<i>Hydromantes imperialis</i>	92	30.29	29.98	0.671	90	0.504	0.14
<i>Hydromantes supramontis</i>	72	28.70	28.80	−0.240	70	0.811	−0.06
<i>Hydromantes flavus</i>	38	29.58	28.50	1.360	36	0.182	0.47
<i>Hydromantes ambrosii</i> <sup>a</sup>	78	25.31	25.44	−0.749	75	0.456	−0.17
<i>Hydromantes italicus</i>	94	25.00	24.94	0.166	92	0.869	0.04
<i>Hydromantes strinatii</i>	270	25.92	25.95	−0.122	268	0.903	−0.01
<i>Hydromantes genei</i> <sup>a</sup>	95	30.54	29.63	−0.749	93	0.456	−0.15

*N*, sample size; in bold, significant differences after sequential Bonferroni's correction

<sup>a</sup> Data analyzed using a mixed model, to take into account potential differences among subspecies, instead of using standard *t* test

best model (Table 3). This model suggests that CVSD was highest in species with complex courtship ( $F_{1,25} = 24.5$ ,  $P < 0.0001$ ), and explained a satisfactory amount of variance ( $R_{\text{adj}}^2 = 0.47$ ). All candidate models representing the other competing hypotheses showed very limited support ( $w < 0.02$ ) and explained a limited amount of variance (Table 3). All models representing the combined effects of multiple variables (included the model representing hypothesis e) showed higher AICc values than their simpler nested models (Appendix S2), and were thus not included among the set of candidate models (Richards et al. 2011).

To test for the support of the aquatic phase hypothesis, we built two models relating the absolute value of CVSD to the length of aquatic phase of adults or larvae. None of these models had support by the data. Both the model considering the length of aquatic larval phase (AICc = 26.9), and the model considering the length of

**Table 3** Candidate models explaining sexual dimorphism for the number of caudal vertebrae in Urodela

Hypothesis	K	AICc	$R_{\text{adj}}^2$	<i>w</i>
(b) Courtship	2	28.4	0.47	0.983
(a) Body-size dimorphism	2	37.0	0.21	0.013
(c) Ornamentation	2	40.4	0.09	0.002
(−) Null model	1	41.5	0.00	0.001

K, number of parameters in the model; AICc, Akaike's information criterion corrected for small sample size;  $R_{\text{adj}}^2$ , adjusted  $R^2$ ; *w*, AIC weight of the model

aquatic phase of adults (AICc = 27.3) showed an AICc higher than the null model (AICc = 25.0) and did not explain the variation in CVSD (for both models,  $R_{\text{adj}}^2 = 0.0$ ). Results were identical if we set length of aquatic phase = 0 in species in which only females enter in breeding wetlands (AICc = 27.3,  $R_{\text{adj}}^2 = 0.0$ ).

We detected significant CVSD in Salamandridae only (Table 2). Furthermore, the best model supported the courtship hypothesis, and the most complex courtships are found in newts (i.e., aquatic salamandridae) (Wiens et al. 2011). We therefore repeated analyses considering salamandridae only (19 species belonging to eight genera). The results of the analysis considering salamandridae only were very similar to the results of the analysis including all species (Appendix S3).

In the previous analyses, we used Cohen's  $d$  as dependent variable, but for the majority of species CVSD was not significant. In order to assess whether including species with non-significant CVSD affects our conclusion, we repeated the global analysis by setting Cohen's  $d$  to zero in all species in which CVSD was not significant. This analysis confirmed the conclusions of the previous ones (Appendix S4).

## Discussion

In this study, we used an information-theoretic approach to assess processes that may promote sexual dimorphism in the number of caudal vertebrae in Urodela. The explicit comparison of a priori models, combined with the use of phylogenetic comparative methods, allowed us to evaluate the relative support of multiple hypotheses, and suggested that the evolution of complex mating behaviours was the most likely determinant of the evolution of CVSD.

CVSD was not widespread in Urodela; after multiple test correction, we detected CVSD in 19 % of analyzed species, all these species belonged to two genera of newts (*Ommatotriton* and *Lissotriton*), and in all these species males were the sex with more caudal vertebrae (Table 2). *Ommatotriton* and *Lissotriton* are among the newts with more complex courtship behaviours. Our analyses suggest that courtship is the most likely determinant of CVSD; the support of the courtship hypothesis was overwhelming, compared to the other hypotheses tested (Table 3, Appendix S2, Appendix S3). During courtship, male newts use tail in a series of display behaviours which serve to attract and stimulate potential mates (e.g., fan, whip, flick) through both visual and chemical stimuli (Griffiths 1995; Wiens et al. 2011). Sexual selection may therefore favour males with morphological characters enhancing displays: a tail with more vertebrae may be longer and more visible, or more flexible. Sexual selection may drive the evolution of multiple morphological traits, and in Salamandridae courtship behaviour is also involved in the evolution of other dimorphic characters, such as dorsal crests (Wiens et al. 2011). It should be remarked that strong CVSD occurred also in species (e.g., *Lissotriton italicus*) with complex courtship but lacking dorsal crests. For small

bodied species such as *L. italicus* the presence of dorsal crests may limit swimming capability, and natural selection has probably led to the loss of this costly secondary character (Wiens et al. 2011). Sexual dimorphism is often less pronounced in smaller species (Dale et al. 2007; Stephens and Wiens 2009), and the selective cost of CVSD for swimming may be smaller than the cost of dorsal crests. Actually, we found no support for the hypothesis that natural selection limits CVSD in more aquatic species (aquatic phase hypothesis), as dimorphism was not less in species spending more time in water.

Nevertheless, the complexity of courtship behaviours does not fully explain the occurrence of CVSD. In newts belonging to the genus *Triturus*, we detected no CVSD despite the very complex courtships they display. In fact *Triturus cristatus* females had slightly more caudal vertebrae than did males, although this difference was not significant after Bonferroni's correction (Table 2). Clearly, courtship behaviours can determine the evolution of multiple morphological characters, and CVSD is only one of the traits that may be targeted by sexual selection. Other morphological characters may be more important for these large-bodied newts, in which some of the most prominent courtship behaviours involve the display of tall, denticulated dorsal crests (Wiens et al. 2011). In other words, complex courtship probably drives the evolution of both dorsal crests and tail morphology, but the evolution of these two traits is not necessarily linked, as shown by the limited support of the ornamentation hypothesis in our models (Table 3, Appendices S3, S4).

The support of the behaviour hypothesis was strong, compared with the other hypotheses (Table 3), and we did not find support for any model combining multiple hypotheses (Appendix S2), suggesting courtship behaviour as the major determinant of CVSD. The sample size used for phylogenetic analyses was not large (27 species), and this might have limited statistical power, reducing the support of models including multiple independent variables. On the other hand, our analysis was based on morphological data from >2,000 individuals, and covered about 75 % of the species currently recognised for European Urodela (Speybroeck et al. 2010) (Appendix S1), plus two Asian species (genus *Ommatotriton*), suggesting that our work accurately reflects the situation within our study area. Additional sampling, including for example other families and Asian or American species, may certainly provide a more complete picture of the evolution of CVSD across Urodela.

For most of species, analyzed individuals come from multiple populations, sometime living under different climatic conditions. In principle, climate may influence sexual dimorphism across populations within species (Ficetola et al. 2010). Nevertheless, we do not expect that climatic

differences among populations would bias our results because, for all study species, analyzed males and females lived under similar climates (see Appendix S5). Furthermore, our analyses aimed at the identification of evolutionary forces determining CVSD at the interspecific level, and intraspecific variation is likely much smaller than the variation among species. Nevertheless, future studies addressing intraspecific variation can shed light on processes determining sexual dimorphism at finer scale than the one considered here (Blanckenhorn et al. 2006).

Our work focused on CVSD; evaluating the processes determining sexual dimorphism for body size was beyond the aims of this study. However, in this analysis sexual dimorphism for body size was included as a potential predictor of CVSD: the body-size dimorphism hypothesis predicted that CVSD would occur only as a by-product of mechanisms determining body size dimorphism. Sexual dimorphism for body size is usually caused by the interplay between fecundity, sexual and natural selection. The available data suggest that, when fecundity selection is more important than the other selective forces in determining body size differences, females tend to be larger than males (Malmgren and Thollessen 1999; Shine 2000; Stephens and Wiens 2009; Zhang and Lu 2012). On the other hand, when sexual selection prevails males are often larger than females, and allometric relationships between the two sexes often emerge (Dale et al. 2007; Stephens and Wiens 2009; but see also Ceballos et al. 2012). In caudate amphibians body size can be sexually dimorphic in different ways. In several species females are larger (dimorphism likely caused by fecundity selection) (Malmgren and Thollessen 1999; Ficetola et al. 2010), but there are also species in which males are larger (e.g., *Ommatotriton* and *Euproctus platycephalus*) and species without noticeable body size differences (Bovero et al. 2003; Ivanovic et al. 2008), suggesting a different importance of fecundity and sexual selection across species. Conversely, sexual selection seems to have the major role in determining the other dimorphic traits, and particularly those involved in courtship, such as the features of tails, dorsal crests and limbs (Table 3; Malmgren and Thollessen 1999; Wiens et al. 2011). Overall, in European Urodela body size dimorphism seems to be decoupled from the dimorphism for courtship-related traits, probably because of the different impact of selective forces (Ivanovic et al. 2008; Wiens et al. 2011).

A tight relationship can link the evolution of courtship behaviour with certain morphological traits. In fact, courtship may be linked with the evolution of multiple, very distinct morphological traits in the same taxon (i.e., caudal vertebrae: this study; dorsal crests: Wiens et al. 2011). Although the relationship between courtship and sexual dimorphism may seem obvious, few studies have addressed this question within a phylogenetically-explicit

framework (see Wiens et al. 2011), and some analyses suggested decoupling between morphological ornamentation and behaviours (Wiens 2000; Quinn and Hews 2010). It is not clear why the relationship between courtship and sexual dimorphism has been detected only in some of the investigated taxa. The increasing availability of detailed phylogenetic information can allow the investigation of this question over a broader taxonomic range, and the identification of situations where the link between behaviour and sexual dimorphism is particularly important.

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## References

- Allen, C. E., Zwaan, B. J., & Brakefield, P. M. (2011). Evolution of sexual dimorphism in the Lepidoptera. *Annual Review of Entomology*, *56*, 445–464.
- Arakelyan, M. S., Dalielyan, F. D., Corti, C., Sindaco, R., & Leviton, A. E. (2011). *Herpetofauna of Armenia and Nagorno-Karabakh*. Ithaca, NY: SSAR.
- Blanckenhorn, W. U. (2005). Behavioral causes and consequences of sexual size dimorphism. *Ethology*, *111*, 977–1016.
- Blanckenhorn, W. U., Stillwell, R. C., Young, K. A., Fox, C. W., & Ashton, K. G. (2006). When Rensch meets Bergmann: Does sexual size dimorphism change systematically with latitude? *Evolution*, *60*, 2004–2011.
- Borcard, D., Gillet, F., & Legendre, P. (2011). *Numerical ecology withy R*. New York: Springer.
- Bovero, S., Sotgiu, G., Castellano, S., & Giacoma, C. (2003). Age and sexual dimorphism in a population of *Euproctus platycephalus* (Caudata: Salamandridae) from Sardinia. *Copeia*, *2003*, 149–154.
- Carranza, S., Romano, A., Arnold, E. N., & Sotgiu, G. (2008). Biogeography and evolution of European cave salamanders, *Hydromantes* (Urodela: Plethodontidae), inferred from mtDNA sequences. *Journal of Biogeography*, *35*, 724–738.
- Ceballos, C., Adams, D., Iverson, J., & Valenzuela, N. (2012). Phylogenetic patterns of sexual size dimorphism in Turtles and their implications for Rensch's rule. *Evolutionary Biology*. doi: 10.1007/s11692-012-9199-y.
- Dale, J., Dunn, P. O., Figuerola, J., Lislevand, T., Székely, T., & Whittingham, L. A. (2007). Sexual selection explains Rensch's rule of allometry for sexual size dimorphism. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 2971–2979.
- Del Re, A. C. (2012). *compute.es: Compute Effect Sizes. R package version 0.2.1*: <http://CRAN.R-project.org/package=compute.es>.
- Ficetola, G. F., Scali, S., Denoël, M., Montinaro, G., Vukov, T. D., Zuffi, M. A. L., et al. (2010). Ecogeographical variation of body size in the newt *Triturus carnifex*: Comparing the hypotheses using an information-theoretic approach. *Global Ecology and Biogeography*, *19*, 485–495.
- Freckleton, R. P., Cooper, N., & Jetz, W. (2011). Comparative methods as a statistical fix: The dangers of ignoring an evolutionary model. *American Naturalist*, *178*(1), E10–E17.
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *American Naturalist*, *160*, 712–726.

- Frydlova, P., & Frynta, D. (2010). A test of Rensch's rule in varanid lizards. *Biological Journal of the Linnean Society*, *100*, 293–306.
- Griffiths, R. A. (1995). *Newts and salamanders of Europe*. London: Poyser Natural History.
- Grossenbacher, K., & Thiesmeier, B. (Eds.). (2003). *Handbuch der Reptilien und Amphibien Europas. Bd. 4/IIA: Schwanzlurche (Urodela) IIA*. Wiesbaden: AULA-Verlag.
- Gvozdik, L., & Van Damme, R. (2006). *Triturus* newts defy the running-swimming dilemma. *Evolution*, *60*, 2110–2121.
- Hartung, J., Knapp, G., & Sinha, B. K. (2008). *Statistical meta-analysis with applications*. Hoboken, NJ: Wiley.
- Itazawa, Y. (1959). Influence of the environment on the number of vertebrae in fish. *Nature*, *183*, 1408–1409.
- Ivanovic, A., Sotiropoulos, K., Furtula, M., Dzukic, G., & Kalezic, M. L. (2008). Sexual size and shape evolution in European newts (Amphibia: Caudata: Salamandridae) on the Balkan Peninsula. *Journal of Zoological Systematics and Evolutionary Research*, *46*, 381–387.
- Lanza, B., Andreone, F., Bologna, M. A., Corti, C., & Razzetti, E. (Eds.). (2007). *Fauna d'Italia, vol. XLII, Amphibia*. Bologna: Calderini.
- Lanza, B., Arntzen, J. W., & Gentile, E. (2009). Vertebral numbers in the Caudata of the Western Palaearctic (Amphibia). *Atti del Museo Civico di Storia Naturale di Trieste*, *54*, 3–114.
- Lanza, B., Pastorelli, C., Laghi, P., & Cimmaruta, R. (2006). A review of systematics, taxonomy, genetics, biogeography and natural history of the genus *Speleomantes* Dubois, 1984 (Amphibia Caudata Plethodontidae). *Atti del Museo Civico di Storia Naturale di Trieste*, *52*(Suppl), 5–135.
- Lovich, J. E., & Gibbons, J. W. (1992). A review of techniques for quantifying sexual size dimorphism. *Growth, Development, and Aging*, *56*, 269–281.
- Lukacs, P. M., Thompson, W. L., Kendall, W. L., Gould, W. R., Doherty, P. F., Burnham, K. P., et al. (2007). Concerns regarding a call for pluralism of information theory and hypothesis testing. *Journal of Applied Ecology*, *44*, 456–460.
- Malmgren, J. C., & Thollessen, M. (1999). Sexual size and shape dimorphism in two species of newts, *Triturus cristatus* and *T. vulgaris* (Caudata: Salamandridae). *Journal of Zoology*, *249*, 127–136.
- Martins, E. P., & Hansen, T. F. (1997). Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *American Naturalist*, *149*, 646–667.
- Mayr, E. (1972). Sexual selection and natural selection. In B. Campbell (Ed.), *Sexual selection and the descent of man 1871–1971* (pp. 136–208). Chicago: Aldine.
- McDowall, R. M. (2008). Jordan's and other ecogeographical rules, and the vertebral number in fishes. *Journal of Biogeography*, *35*, 501–508.
- McIntire, E. J. B., & Fajardo, A. (2009). Beyond description: The active and effective way to infer processes from spatial patterns. *Ecology*, *90*, 46–56.
- Nöllert, A., & Nöllert, C. (1992). *Die Amphibien Europas*. Stuttgart: Kosmos.
- Orme, D., Freckleton, R. P., Thomas, G. H., Petzoldt, T., Fritz, S., Isaac, N., et al. (2012). *caper: Comparative Analyses of Phylogenetics and Evolution in R. R package version 0.5*: <http://CRAN.R-project.org/package=caper>.
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*, 289–290.
- Quinn, V. S., & Hews, D. K. (2010). The evolutionary decoupling of behavioral and color cues in a multicomponent signal in two *Sceloporus* lizards. *Ethology*, *116*, 509–516.
- R Development Core Team. (2012). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Raffaelli, J. (2007). *Les Urodèles du monde*. France: Penclen.
- Rice, W. R. (1989). Analyzing tables of statistical tests. *Evolution*, *43*, 223–225.
- Richards, S. A., Whittingham, M. J., & Stephens, P. A. (2011). Model selection and model averaging in behavioural ecology: The utility of the IT-AIC framework. *Behavioral Ecology and Sociobiology*, *65*, 77–89.
- Shine, R. (2000). Vertebral numbers in male and female snakes: The roles of natural, sexual and fecundity selection. *Journal of Evolutionary Biology*, *13*, 455–465.
- Speybroeck, J., Beukema, W., & Crochet, P. A. (2010). A tentative species list of the European herpetofauna (Amphibia and Reptilia)—An update. *Zootaxa*, *2492*, 1–27.
- Stephens, P. R., & Wiens, J. J. (2009). Evolution of sexual size dimorphism in Emydid turtles: Ecological dimorphism, Rensch's rule, and sympatric divergence. *Evolution*, *63*, 910–925.
- Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioral Ecology and Sociobiology*, *65*(1), 13–21.
- van der Meijden, A., Chiari, Y., Mucedda, M., Carranza, S., Corti, C., & Veith, M. (2009). Phylogenetic relationships of Sardinian cave salamanders, genus *Hydromantes*, based on mitochondrial and nuclear DNA sequence data. *Molecular Phylogenetics and Evolution*, *51*, 399–404.
- Veith, M. (1994). Morphological, molecular and life history variation in *Salamandra salamandra* (L.). *Mertensiella*, *4*, 355–397.
- Wielstra, B., & Arntzen, J. W. (2011). Unraveling the rapid radiation of crested newts (*Triturus cristatus* superspecies) using complete mitogenomic sequences. *BMC Evolutionary Biology*, *11*, 162.
- Wiens, J. J. (2000). Decoupled evolution of display morphology and display behaviour in phrynosomatid lizards. *Biological Journal of the Linnean Society*, *70*, 597–612.
- Wiens, J. J., Sparreboom, M., & Arntzen, J. W. (2011). Crest evolution in newts: Implications for reconstruction methods, sexual selection, phenotypic plasticity and the origin of novelties. *Journal of Evolutionary Biology*, *24*, 2073–2086.
- Zhang, L., & Lu, X. (2012). Sexual size dimorphism in Anurans: Ontogenetic determination revealed by an across-species comparison. *Evolutionary Biology*. doi:10.1007/s11692-012-9187-2.
- Zhang, P., & Wake, D. B. (2009). Higher-level salamander relationships and divergence dates inferred from complete mitochondrial genomes. *Molecular Phylogenetics and Evolution*, *53*, 492–508.
- Zug, G. R., Vitt, L. J., & Caldwell, J. P. (2001). *Herpetology*. San Diego: Academic Press.