

Sexual dimorphism and age structure of the Montseny newt (*Calotriton arnoldi*)

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Abstract. Patterns of sexual dimorphism and age structure were investigated in two populations of the newt *Calotriton arnoldi*, endemic of the Montseny Massif (NE of the Iberian Peninsula). In contrast to the Pyrenean newt (*Calotriton asper*) sexual dimorphism in the Montseny brook newt is characterized by slightly larger females (60.3 ± 0.3 mm; maximum: 68 mm) than males (59.5 ± 0.2 mm; maximum: 64 mm) and more similar body shape between sexes. Both populations and sexes mature at the same age (3 years), show the same age structure and achieve similar longevity (8-9 years). Comparing our results with the framework of the variation of life-history traits in *Calotriton*, the Montseny newts exhibit fast sexual maturity and short longevity. Curiously, we have found a lack of covariation between age at sexual maturity, longevity and total body size in the populations of *Calotriton* species. Only in males, age at sexual maturity seems to be affected by altitude, but in an unexpected way: sexual maturation is delayed in populations at low altitudes. Moreover, the age at sexual maturity does not differs between the populations where immatures are terrestrial vs. those where they remain aquatic. Our results suggest that life-history traits in *Calotriton* newts could be determined by selective factors that play their role at small geographic scale.

Keywords: age structure, *Calotriton arnoldi*, skeletochronology, morphology.

Introduction

The salamanders and newts of the family Salamandridae have diversified in an array of ecological forms, ranging from fully terrestrial, to aquatic and semiaquatic species that live in ponds and streams (Wells, 2007). This evolutionary radiation has usually involved changes in body size and shape, and several differences in the tongue morphology and the hyolingual apparatus, as well as the lung development. These variations are related to the life-style and specialization of prey capture in different kinds of terrestrial and aquatic habitats

(Özeti and Wake, 1969). Another example of the degree of salamandrid diversification is the evolution of different modalities of reproductive behaviour (Salthe, 1967; Arntzen and Sparreboom, 1989) probably linked to the habitats used during the courtship. This also could have promoted the development of different morphological patterns of sexual dimorphism (Halliday, 1990; Houck and Arnold, 2003; Wiens et al., 2011). Life-history strategies have been also diversified in many aspects, parallel to the radiation of this family, including parameters that can be the target of the natural selection, as for example the age at sexual maturity and longevity.

Local adaptation to different environmental conditions in species inhabiting large altitudinal and wide geographical ranges is one source of age structure variation in salamandrid species (Díaz-Paniagua and Mateo, 1999; Marunouchi et al., 2000; Denoël, 2001; Miaud and Guillaume, 2005; Olgun et al., 2005; Üzum and Olgun, 2009). Congruently, species living in similar main habitats (i.e. terrestrial, fossorial, reophilous and lentic) often exhibit similar life-histories (Wells, 2007). For instance, physiological constraints to activity, exposition to pre-

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dition or availability of trophic resources differ between terrestrial and aquatic habitats, thus shaping part of the variation between species.

Age at sexual maturity, longevity and age structure are key demographic parameters that have been investigated in full terrestrial, fossorial, semiaquatic lentic and lotic species (Lima et al., 2000; Marunouchi et al., 2000; Olgun et al., 2001; Bovero et al., 2003; Maletzky et al., 2004; Miaud and Guillaume, 2005; Seglie et al., 2010; Angelini et al., 2015). Among salamandrids, *Calotriton* newts are two species of stream dwellers, living in the Iberian Peninsula and closely related to the *Triturus* genus (Carranza and Amat, 2005). The Montseny brook newt (*C. arnoldi*) diverged approximately 1–1.5 million years ago from the Pyrenean brook newt (*C. asper*), probably as a result of the allopatric isolation of its common ancestor in the Massif of Montseny, the southernmost limit of genus range (Carranza and Amat, 2005). The Montseny brook newt is distributed in two separate areas situated in the east (three populations) and the west (four populations) of the Tordera River (Amat and Carranza, 2006). Remarkably, the analysis of mitochondrial DNA and the patterns of coloration identified differences between eastern and western populations (Valbuena et al., 2013), indicating that they have also been isolated for many generations. *C. arnoldi* inhabits small streams at medium altitude (600–1300 m) characterized by warmer and xeric conditions (i.e., Mediterranean climate) (Amat and Carranza, 2006). In contrast, *Calotriton asper* lives in cold waters in most part of the Pyrenees within a wide altitudinal range, from 190 to 3300 m (García-París et al., 2004), mainly in streams and rarely in aquatic caves and lakes (Clergue-Gazeau and Martínez-Rica, 1978). The most remarkable ecological difference between the two *Calotriton* species is that after the metamorphosis, *C. arnoldi* immatures remain in the water (Amat and Carranza, 2006). In contrast, *C. asper* undergoes an ontogenetic change of habitat. After metamorphosis, immatures live in terrestrial habitats and after

reaching to the sexual maturity, young adults return to live in reophilous habitats (García-París et al., 2004; Montori et al., 2008). The only modification of this pattern is the retention of a full aquatic life in immatures living in lentic habitats, like the high altitude lakes in the Pyrenees. Therefore, the study of the Montseny brook newt biology is interesting for the comprehension of the implications of the ontogenetic changes of habitat, in the framework of the adaptive radiation of salamandrids.

The first goal of this study is to report first data on sexual dimorphism, age at sexual maturity, longevity and age structure in the Montseny brook newt. To account for the intraspecific variation, we analyse the morphological and genetic differences between the two populations of *C. arnoldi* (eastern and western) (according to Valbuena et al., 2013). Moreover, we compare several life-history traits in the Montseny newt with those of the sister species *C. asper*.

Material and methods

Populations and study sites

Field sampling was conducted in the Montseny Natural Park in spring of 2007. We refer to the sampled populations as western and eastern, following the geographic structure of the species (Carranza and Amat, 2005). The exact locations were not provided in order to protect the populations of this critically endangered species. The sexual dimorphism and age structure in the *C. arnoldi* populations were studied in an eastern stream and two tributaries of a western stream. These two sampled tributaries were considered as part of the same population for morphological and skeletochronological analyses, because they are well connected between them and shares identical habitat. The eastern stream sampled flows between 1150–1200 m, facing south in a beech forest (*Fagus sylvatica*), while the western stream is placed in an oak forest (*Quercus ilex*) facing north at 750 to 820 m.

Morphometric analysis

Eight linear morphometric measurements, using a digital calliper with 0.1 mm accuracy, were obtained from the individuals captured during samplings – 98 individuals (48 males and 50 females) and 65 individuals (38 males and 27 females) for the eastern and western populations, respectively. The following morphometric variables were measured: the SVL, the head length and width, the fore and hind limb length, the limb interval (distance between the closest insertion points of limbs at ventral right side), the tail length

and its maximum height. Individuals were sexed considering the distinctive morphology of the cloaca in each sex and presence of ova in the ovaries observed through the translucent vent (Carranza and Amat, 2005), to assess sexual maturity in females.

Morphometric variables were tested for normality and homocedasticity and log10 transformed. We tested for sexual differences, taking into account the effect of geographic factors (eastern vs. western populations), on SVL using Wilk's lambda from two-way ANOVA and for the other morphometric variables by means a two-way MANCOVA, using SVL as a covariable. In addition, we performed a principal component analysis (PCA) including all the morphometric variables. All these analyses as well the following specified in other methodological sections were performed using Statistica 4.5.

Skeletochronological age estimation

A subsample of newts captured for the morphological analyses (46 from the western population and 42 from eastern one) was used to estimate the age of individuals using skeletochronological methods (Smirina, 1972). The largest toe of the left hind limb was removed and stored in 70% alcohol. The phalanges were decalcified in 3% nitric acid for 5 min. After this procedure, cross sections (12–14 μm) were obtained with a freezing microtome and stained with Ehrlich's hematoxylin. The age of each individual was determined by counting the LAGs in the diaphysis of the periosteal bone using a light microscope at magnifications of 200 \times and 400 \times . Skeletochronological data were used to determine the age at maturity, the longevity, and the age structure of each population. Sexual and population differences in the age structure were analysed by means of Kolmogorov-Smirnov test in order to compare the shape of the age distributions. Relationships between the age and the body size were estimated using Pearson correlation. In addition, two-way ANCOVA was performed to test for sexual and population differences in SVL in relation to age as covariate.

Age and body size variation in Calotriton

The data available on age at maturity and longevity were compiled in seven populations from three different studies (Montori, 1988, 1990; Miaud and Guillaume, 2005; Oromí et al., 2014) comprising one lacustrine and six reophilous

populations placed from 490 to 1886 m of altitude. Miaud and Guillaume (2005) only provided minimum and maximum total length instead of SVL. Therefore and for comparative purposes we used total length (SVL plus tail size) as a measure of body size. We tested for the effect of altitude and immature terrestriality on the age at sexual maturity and minimum total length, separately for each sex using ANOVA and Pearson correlation. In addition, correlation between the age at sexual maturity and longevity was also tested.

Results

Sexual dimorphism

The males and females being slightly larger than 51–53 mm, were sexually mature. Differences in SVL were found between sexes ($F_{1,159} = 4.123$, $P = 0.043$), being females slightly larger than males (mean \pm SE: 60.3 \pm 3.0 and 59.9 \pm 2.2). Eastern and western populations were not significantly different in body size ($F_{1,159} = 0.025$, $P = 0.874$) and showed the same pattern of sexual dimorphism for this morphometric variable ($F_{1,159} = 3.382$, $P = 0.067$). MANCOVA showed significant differences between sexes ($\Lambda_{7,152} = 0.585$, $P < 0.0001$) and populations ($\Lambda_{7,152} = 0.162$, $P < 0.0001$), but no significant interaction between these two factors ($\Lambda_{7,152} = 0.376$, $P = 0.376$). A post-hoc test for unequal sample size revealed differences between sexes ($P < 0.001$) in all the variables with the only exception of the limb interval (table 1).

The first two factors from the PCA accounted for 62.1% of the total variance, showing a clear sexual pattern of morphological covariation (table 2). The first PC was well defined by the

Table 1. Descriptive statistics (mean and standard deviation) of the morphometric variables used in the multivariate analyses of males and females, and significance of MANCOVA's post-hoc test.

Variable	Males $n = 86$	Females $n = 77$	P
Head length	13.2 \pm 0.9	12.0 \pm 0.8	<0.0001
Head width	10.5 \pm 0.7	9.5 \pm 0.6	<0.0001
Forelimb length	15.1 \pm 0.9	14.5 \pm 0.8	<0.0001
Hindlimb length	17.5 \pm 1.0	16.0 \pm 0.8	<0.0001
Limb interval	31.0 \pm 2.6	30.7 \pm 2.7	0.586
Tail length	41.9 \pm 2.4	44.0 \pm 3.3	<0.0001
Tail high	7.9 \pm 0.7	5.2 \pm 0.6	<0.0001

Table 2. Weight coefficients of eight morphometric measurements for the two first factors from PCA, including eigenvalues and percentage of accumulated variance.

Variable	1st Root	2nd Root
SVL	-0.399	0.736
Head length	-0.761	-0.097
Head width	-0.722	-0.216
Forelimb length	-0.784	0.056
Hindlimb length	-0.794	-0.023
Limb interval	-0.305	0.566
Tail length	0.004	0.809
Tail height	-0.829	-0.313
Eigenvalues	3.297	1.677
% Variance	41.2	20.9

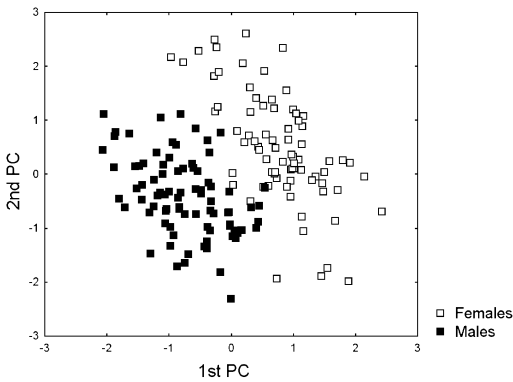


Figure 1. Scatterplot of scores of the *Calotriton arnoldi* samples in the first two factors of the principal component analysis: females (open squares) and males (black squares).

length of limbs, tail high and head size with high negative loads, and on the opposite by the tail length. The second PC was defined as a contrast between the high positive loads of SVL, limb interval and tail length, and the high negative load of tail high. The representation of individual scores on these two first PC, showed few overlap between males and females, indicating a clear sexual differentiation (fig. 1). In fact, females usually have smaller heads, shorter limbs and longer tails, while males are characterized by shorter, but higher tails, and bigger heads (table 1).

Skeletochronological analysis

The toe cross-sections did not show resorption lines and no rapprochement of the bone was

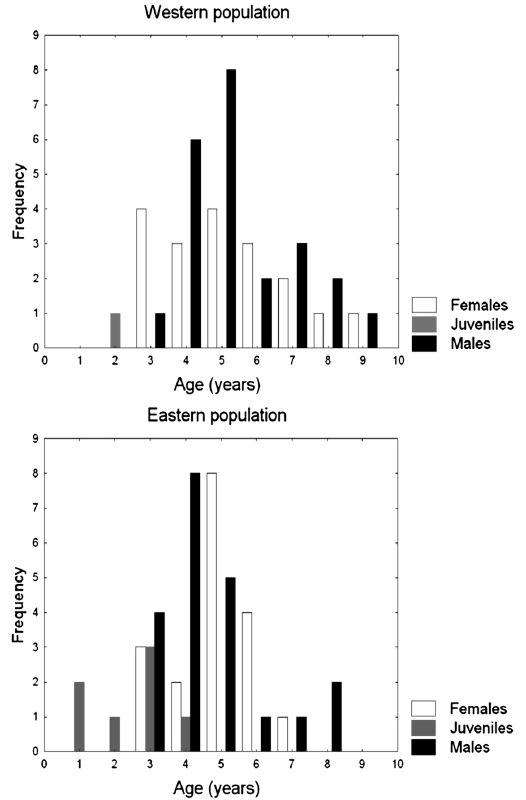


Figure 2. Age structure by sexes for *Calotriton arnoldi* newts from eastern and western populations.

observed. Thus, estimates of lines of arrested growth (LAGs) were considered accurate. The ages of immatures ranged from 1 to 4 years in the eastern population, while the only immature from the western population was two years old. The earliest males and females from the eastern and western populations reached sexual maturity at 3 years. Considering the highest age of immatures, adulthood can be delayed up to 4 years. The median age (5 years) was the same for sexes in both populations (fig. 2), while lifespan differed between populations. In the eastern population, the lifespan was similar for males and females (9 years), but in the western one it was 8 years for males and 7 years for females. The age structure did not differ between sexes within each geographical area (western and eastern) and between geographical areas (Kolmogorov-Smirnov tests, $P > 0.10$). The age was correlated with the SVL in the adult

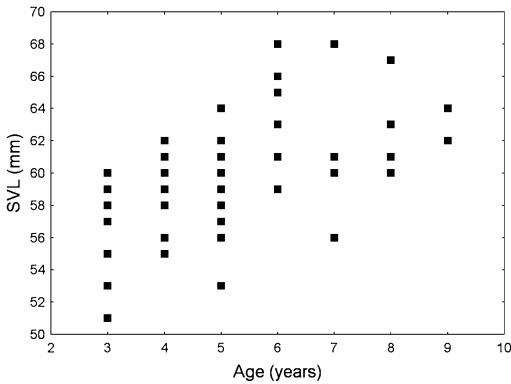


Figure 3. Age and body size (SVL) relationships for the whole sample of *Calotriton arnoldi*.

newts in the total sample ($n = 80$, $r = 0.552$, $P < 0.001$, fig. 3). In the western population age was correlated with SVL for males ($n = 21$, $r = 0.464$, $P = 0.034$) and females ($n = 18$, $r = 0.759$, $P < 0.001$), as well as in the eastern population (males $n = 23$, $r = 0.585$, $P = 0.003$, females $n = 18$, $r = 0.587$, $P = 0.010$). The SVL corrected for age was not different between sexes (ANCOVA $F_{1,75} = 3.189$, $P = 0.078$) but varied between populations (ANCOVA $F_{1,75} = 5.196$, $P = 0.025$), without interaction between these two factors (ANCOVA $F_{1,75} = 2.496$, $P = 0.118$). The newts of the western population showed larger SVL relative to its age than those of the eastern population.

Age and body size variation in *Calotriton*

The analyses of the eight *Calotriton* populations showed that the average age at sexual maturity was not correlated to maximum longevity in both sexes (males, $r = 0.523$, $P = 0.183$ and females, $r = 0.539$, $P = 0.167$). In addition, we did not find significant correlation between the total length and the age at sexual maturity in males ($r = 0.643$, $P = 0.085$) and females ($r = -0.422$, $P = 0.298$). Altitude was negatively correlated with the age at sexual maturity in males ($r = -0.905$, $P = 0.002$), but not in females ($r = -0.479$, $P = 0.229$). On the other hand, two-way ANOVA testing the effects of

sex and a terrestrial vs. aquatic immature phase, showed not significant effect on the age at sexual maturity in males (ANOVA $F_{1,7} = 3.816$, $P = 0.091$) and females (ANOVA $F_{1,7} = 3.966$, $P = 0.086$).

Discussion

Sexual dimorphism in the Montseny brook newt is characterized by few differences on body size between the two studied populations. In contrast, the Pyrenean brook newt shows a large geographic variation in body size (Guerrero-Campo, 1995; García-París et al., 2004) and patterns of sexual dimorphism in this species seems to be complex, varying among populations (Serra-Cobo et al., 2000; García-París et al., 2004). This species has a large geographic range and lives in wide diversity of habitats, contrasting with the very restricted range and habitat specialization of the Montseny species. In amphibians, sexual size dimorphism could be due to differences on body size at sexual maturity, rate of growth and lifespan (Zhang and Lu, 2013). Our results indicate that both sexes mature at similar size without notable variation on the age. Therefore, the small differences found among growth rates between males and females, could have produced the subtle sexual dimorphism of body size in *C. arnoldi*.

Both *Calotriton* species have the same pattern of sexual dimorphism in body shape characterized by larger heads and shorter and higher tails in males. This pattern can have been produced by the development of a reproductive behaviour adapted to fast-flowing waters and to increases biting ability in this sex (Montori, 1988; Serra-Cobo et al., 2000). The aggressive biting among males has been occasionally observed in *C. arnoldi* (F. Amat, pers. obser.) and *C. asper* (Lengvenus and Parzefall, 1992). Although *C. arnoldi* as well as the Pyrenean species seems to be not territorial (Guillaume and Cavagnara, 1998), biting could be used by males to compete for access to females, as found for example in plethodontid salamanders

(Marvin, 2009). However, males of *Calotriton* perform a courtship using their tail to retain females (Thiesmeier and Hornberg, 1990) as adaptation to internal fertilization in reophilous environments. This behaviour can be functionally achieved by the development of shorter and higher tails in males, thus increasing their ability to subject females (García-París et al., 2004).

We found a few interpopulational differentiations in the age structures, being only the longevity, lifespan and age-size relationship slightly different. This is due to the small geographic range of the species distribution and the general similarity in habitat of the Montseny species. Furthermore, the predominant use of interstitial spaces in rocky streambeds, combined with little activity on the surface, can have reduced largely the influence of external conditions as altitude or habitat on those traits.

Calotriton newts show a large demographic variation in terms of age at sexual maturity and longevity (table 3) in comparison with other species (see for example Marunouchi et al., 2000). The Montseny brook newt matures earlier than many of the Pyrenean populations with the only exception of those in the Prepyrenean massif of the Cerdanya (Montori, 1988).

Both populations share the influence of the Mediterranean climate, more intensely in the case of *Calotriton arnoldi*. On the other hand, the longevity in the Montseny species is limited, only comparable with that found in two populations of the western Pyrenees (Barranco and Ibon de Acherito, Oromí et al., 2014), submitted to a very distinct climatic regime. Unexpectedly, the analyses failed to reveal a relationship between age at sexual maturity and longevity, detected in other species (Dolmen, 1983; Cea-tano and Castanet, 1993; Miaud et al., 2000; Marunouchi et al., 2001) and predicted by life-history theory (Roff, 2002), but this may be partially related to small sample size. Moreover, minimum total body size does not increase with the age at sexual maturity. This morphometric measurement could not be a good descriptive of the body size, especially in species with sexual dimorphism in tail length like *Calotriton* newts. The delay of the age at sexual maturity with the increasing of the altitude is commonly encountered in many amphibian populations (Morrison and Hero, 2003; Cvetkovic et al., 2009). Surprisingly, we found an opposite pattern in males and lack of altitudinal effect in females. Our analysis also rejected a significant effect of the

Table 3. Data of the total length (TL: SVL plus tail length), age at sexual maturity and longevity in *Calotriton asper* and *Calotriton arnoldi*. References: 1, Montori, 1988, 1990; 2, Miaud and Guillaume, 2005; 3, Oromi et al., 2014 and unpublished data.

Species	Population	Altitude (m)	Sex	TL range (mm)	Maturity (years)	Longevity (years)	Ref.
<i>C. asper</i>	Cerdanya	1450	males	90-130	3	26	1
			females	95-153	3	36	
	Aude	490	males	110-130	9	24	2
			females	70-150	9	26	
	Flumen	1400	males	90-120	6	22	2
			females	90-110	8	29	
	Neouvielle	2350	males	–	5	28	2
			females	–	5	20	
	Caralp	550	males	110-135	7	16	2
			females	105-130	7	19	
Ibón de Acherito	1886	males	94-121	4	12	3	
		females	88-127	4	9		
Barranco de Acherito	1485	males	86-124	4	7	3	
		females	105-145	4	8		
<i>C. arnoldi</i>	Eastern Montseny	1150	males	93-110	3	9	this study
			females	93-115	3	9	
	Western Montseny	750	males	99-106	3	8	
			females	93-102	3	7	

ecology of immatures on the age at sexual maturity, despite that terrestrial and aquatic environments can be likely very different in aspects relevant for growth and survivorship.

Therefore, the studied parameters in *Calotriton* populations and its interrelationships do not follow the expected patterns found in other species of newts. Other causal factors as for example microclimatic variation, differences on predatory pressure, risk of catastrophic declines or competence between individuals, acting at small scale could also play an important role.

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