

## Ontogenetic pattern change in amphibians: the case of *Salamandra corsica*

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Submitted on: 2011, 27<sup>th</sup> February; revised on: 26<sup>th</sup> July; Accepted on: 16<sup>th</sup> September.

**Abstract.** Ontogenetic, post-metamorphic pattern development is a rarely studied topic in amphibian science. As there are indications that the pattern of *Salamandra corsica* might expand over time, digital image analyses were applied in order to measure several phenotypical variables which were related to the snout vent length. Results show a significant increase of patches which change to irregular shapes while SVL increases. Digital image analysis is identified as a suitable tool to explore pattern shape and change in general, while the documented pattern development in *S. corsica* might be one of the first quantified cases of post-metamorphic ontogenetic pattern change in amphibians.

**Keywords.** *Salamandra corsica*, digital image analysis, ontogeny, colour pattern development.

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Coloration in the amphibian skin is composed of structural different xanthophores, iridophores and melanophores, which usually develop during the larval stage (Pederzoli et al., 2003; Vitt and Caldwell, 2009), after which colour patterns typically stabilize shortly after metamorphosis (e.g. Klewen, 1991). However, there are several reports of ontogenetic pattern change, i.e. post-metamorphic change in the colour pattern over time in several species of amphibians (e.g. Lantz, 1953; Bogaerts, 2002). While few in number, the majority of experimental pattern development research has taken place during the early 20<sup>th</sup> century and was mostly based on the relation between background colour and amount of dorsal and ventral patches in members of the genera *Salamandra* and *Triturus* (Kammerer, 1913; Herbst, 1924; Lantz, 1953). Likely due to the rarity of such studies and the fact that most were based on anecdotal evidence, ontogenetic pattern change was not mentioned in several recent reviews on amphibian biology (Duellman and Trueb, 1994; Wells, 2007; Vitt and Caldwell, 2009).

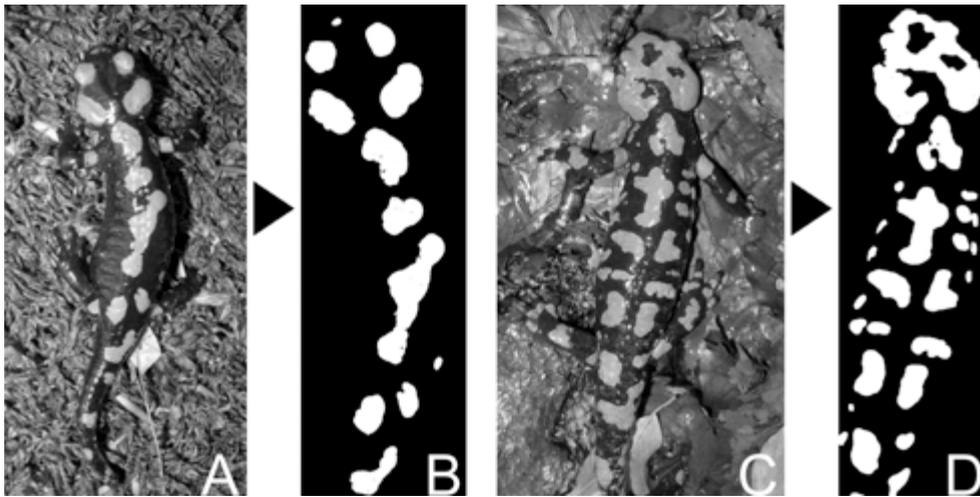
Recent developments in digital image analyses of amphibian phenotypes (e.g. Davis and Maerz, 2007; Vörös et al., 2007) could be especially useful in exploring and quantifying ontogenetic pattern change. These analyses are increasingly used to measure a vari-

ety of morphological and phenotypical features in amphibians such as coloration, pattern and shape (e.g. Relyea, 2004; Davis and Maerz, 2007; Vörös et al., 2007) which can be applied for multiple purposes including evolutionary research and systematics (e.g. Davis and Grayson, 2007; Vörös et al., 2007). Their use permits the quantification of phenotype traits, such as amphibian colour patterns, which have been used widely in taxonomy, but traditionally often have been based on anecdotal evidence only.

Members of the genus *Salamandra* occur over most of Europe and the Mediterranean area, ranging south- and eastwards into North Africa and the Near East (review in Thiesmeier, 2004). While a high number of (subspecific) taxa have been described during the last century especially within *Salamandra salamandra*, the general taxonomy of the genus is unresolved (Steinfartz et al., 2000). Traditionally, the majority of taxa belonging to the 'Fire salamanders' (as opposed to the 'Alpine salamanders') have been described based on their diagnostic yellow colour pattern composed of xanthopores (Eiselt, 1958). However, data regarding colour patterns is often restricted to the type series of the taxon originating from a single locality, while only limited research has been performed concerning intraspecific pattern colour variation within *Salamandra* (but see e.g. Bosch and López-Bueis, 1994; Bogaerts, 2002; Donaire Barroso et al., 2009). There are indications of ontogenetic pattern change in several *Salamandra* taxa (Eiselt, 1958; Mutz, 1992; Bogaerts, 2002). Contrasting statements exist on the pattern of one of these taxa, *Salamandra corsica*. Savi (1838) described the species as being characterized by only sparse yellow coloration, while Eiselt (1958) suspected the yellow pattern to expand and merge during development after metamorphosis. Consequently, qualitative analysis of the pattern of *S. corsica* might both provide an example of rarely studied pattern change, while resolving pending issues on the morphology of the species.

In order to gather data on dorsal pattern of *S. corsica*, fieldwork was conducted in Forêt de Vizzavona (Haute-Corse, 42°06'N, 9°07'E), Corsica at approximately 1200m altitude, from the 28<sup>th</sup> of April until the 2<sup>nd</sup> of May 2005. The study site is a *Fagus sylvatica* forest on a granite slope with a thick leaf litter layer and conclusively little undergrowth apart from several *Asplenium* species and *Cyclamen hederifolium*. A small brook crosses the area which is used for reproduction by *S. corsica*. Dorsal photos were made with a Nikon Coolpix E4600 of a total of 35 individuals which were immediately released after measuring their snout vent length in mm (SVL; the distance from the tip of the snout to the posterior margin of the cloaca).

Due to undulations of the limbs and tail, images of individual *S. corsica* were cropped in Adobe Photoshop CS2 to include only the dorsum and head defined as the Region Of Interest (ROI). The Noise Filters 'Despeckle' (detects the edges in an image and blurs all of the selection except those edges) and 'Median' (within the radius of a pixel those pixels with similar brightness are selected while discarding pixels that differ too much from adjacent pixels; the centre pixel is replaced with the median brightness value of the searched pixels) were used to facilitate subsequent selection of the ROI against heterogeneous backgrounds. Using the 'Magic Wand Tool' with a tolerance of 50, yellow patches (defined as areas of xanthopores) on the dorsum and head, as well as lateral patches continuing onto the dorsum were selected and saved as a separate image (Fig. 1). Pixel surfaces of the ROI, area of yellow patches, perimeter of yellow patches and circularity (CI) were calculated with the freeware ImageJ (Ferreira and Rasband, 2011). The area of yellow patches was converted to a percentage of the ROI area. Patch circularity was defined as



**Fig. 1.** Example of unprocessed images (A: juvenile *Salamandra corsica*, C: adult *Salamandra corsica*) to processed images showing the selected patch area on the Region Of Interest of the same individuals (B,D).

$CI = 4\pi(\text{area}/\text{perimeter}^2)$  in which a CI of 1 represents a perfect circle while 0 is an infinitely elongated polygon. Furthermore, the number of yellow patches was visually summed. Statistics were computed in SPSS16. A Shapiro-Wilk test was used to investigate normality. Subsequently, Spearman’s Rank Correlation Test was used to examine the strength of relations between SVL, CI, number of patches and area of patches. Significant relations between variables were tested with a  $P < 0.05$ .

Descriptive statistics on the measured variables (N = 35) are given in Table 1. SVL displayed a strong correlation with circularity (Spearman’s  $r_s = -0.889$ ,  $P < 0.0001$ ) and the number of patches ( $r_s = 0.783$ ,  $P < 0.0001$ ). Additionally, circularity was strongly correlated with the number of patches ( $r_s = -0.864$ ,  $P < 0.0001$ ). Although a weak correlation was recovered between SVL and the area of patches ( $r_s = 0.254$ ) the relation proved to be insignificant ( $P = 0.141$ ).

**Table 1.** Descriptive statistics on the phenotypical variables derived from digital image analyses and the snout vent length of thirty-five *Salamandra corsica*.

Variable	Mean	Minimum	Maximum	SD
Circularity (index)	0.0000046	0.0000023	0.0000086	0.0000017
Area of patches (%)	29.73	15.68	49.78	10.04
Number of patches	19.54	11	35	7.04
SVL (mm)	74.71	32.43	100.01	21.83

The current results show a significant positive relationship between SVL and number of yellow patches, and a negative relationship between SVL and patch circularity. While

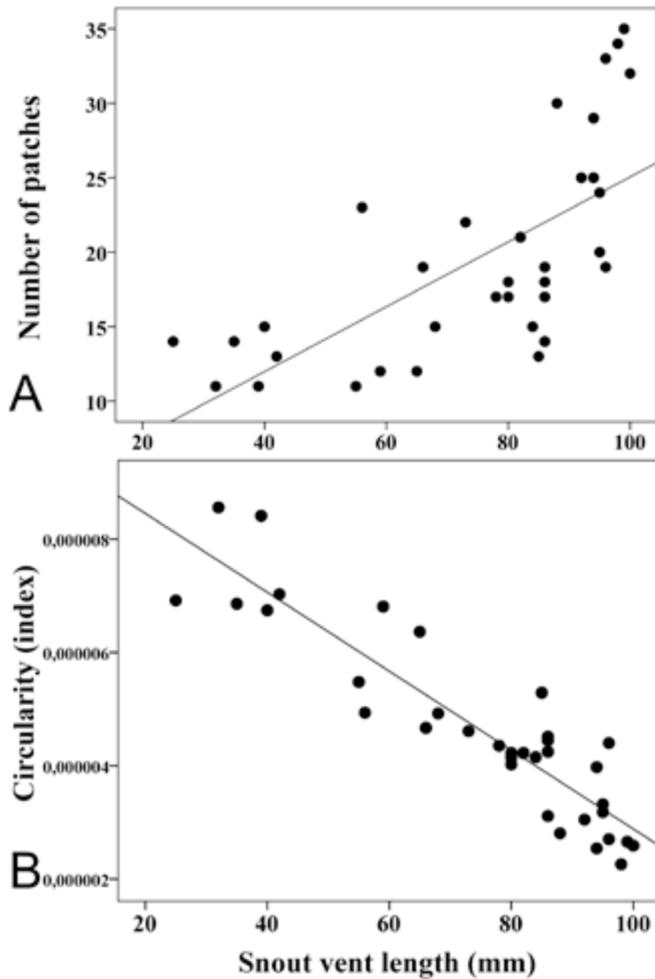


Fig. 2. Correlations between snout vent length and number of patches (A) and circularity (B).

the area of yellow patches does show a weak relation with SVL, this relationship is not significant. This could be an effect of low sample size. On the other hand, the relative area of yellow patches might increase only slightly or remain approximately equal when patches ontogenetically change to a more irregular shape. Visual inspection of the photographed individuals indeed showed several cases of merged patches on the head (see also Fig. 1) as described by Eiselt (1958), but the general pattern seems to be highly inconsistent with considerable variation in patch number and area of yellow patches in adults (Fig. 2). Therefore, as displayed by the highly significant relationships ontogenetic pattern development is present in *S. corsica*, but is characterised by substantial intraspecific variation.

Bogaerts (2002) provided photographic evidence on the ontogenetic pattern development in a small sample of south-Iberian *S. s. crespoides* and *S. s. gallaica* over the course of a

few years, while noting that Moroccan *S. algira tingitana* seems to show ontogenetic reduction of xanthophores. A similar tendency of iridophore decrease was suspected by Beukema et al. (2009) for a population of Anatolian *Lyciasalamandra luschani finikensis*. In addition to its relevance for general morphology and systematics, ontogenetic pattern change can be also an important factor within long term population studies based on individual recognition by means of colour pattern. Such studies have been applied on different subspecies of *S. salamandra* (e.g. Kopp-Hamberger, 1998; Carafa and Biondi, 2004), without taking significant changes in pattern into account. Errors could potentially arise when recognition of prior identified individuals fails due to post-metamorphic development, similar to a recent case described for the viperid *Vipera ursinii* (Tomović et al., 2008).

Conclusively, the currently documented pattern development in *S. corsica* might be one of the first quantified cases of post-metamorphic pattern change in amphibians. While according to literature ontogenetic pattern change appears to occur rarely, it is at least present in several taxa within the Salamandridae, and presents an interesting topic for future research.

#### ACKNOWLEDGEMENTS

Jan Beukema and Mark Bakkers are thanked for assistance in the field. Sergé Bogaerts provided valuable discussion on pattern development among *Salamandra* taxa.

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