## ONTOGENETIC INCREASE OF BLACK DORSAL PATTERN IN RANA TEMPORARIA

## ANTONIO RIOBO\*, JORGE REY\*, MARTA PUENTE\*, CALIA MIRAMONTES\*, MIGUEL VENCES\*\*

\*Laboratoria de Anatomia Animal, Facultad de Ciencias, Universidad de Vigo, E-36200 Vigo, Spain \*\*Zoologisches Forschungsinstitut and Museum A. Koenig, Adenaueralle 160, D-53113 Bonn, Germany..

The widely distributed Common Frog, Rana temporaria, shows an important variation in colour and pattern both between and within populations (Arnold and Burton 1978). One conspicuous pattern element are the large dorsal black spots which are mainly found in specimens from montane populations, e.g. in the Alps (Nöllert and Nöllert 1993) and in the Pyrenees (pers. obs.). A similar trend of black pattern increasing with altitude has also been mentioned in the North-American species Rana cascadae (Behler and King 1979). Several melanic or black coloured amphibian species and morphs are known from high altitudes (e.g. Vences et al. 1998). Explanations of this ecological correlate may be found in thermoregulatory aspects and/or in enhanced protection against ultraviolet radiation, but little is known of the evolutionary and ontogenetic mechanisms leading to increased black colouration at high altitudes. On the other hand, the recently recognized trends of global amphibian declines (e.g. Wake 1991; Blaustein and Wake 1995) seem to affect especially montane amphibians. This may be partly due to increasing ultraviolet radiation, which has been demonstrated to be responsible for the death of eggs and embryos of several species (e.g. Blaustein et al. 1994; Pedraza and Lizana 1997). Considering this possible danger for montane amphibians, it is important to study their natural adaptations to high mountain habitats. The aim of the present note is to provide a contribution to the knowledge of ontogenetic pattern variation in a montane Common Frog population.

In July 1998 we studied a large number of Rana temporaria specimens at the Circo de Piedrafita in the central western Pyrenees, Aragon region, Spain, at an altitude of ca. 2200 m in their summer habitat. Captured specimens were measured, and their colouration recorded; finally, they were marked by toe-clipping and released. We classified specimens in 6 classes based on the amount of black dorsal patterning (Fig. 1): (1) back without black spots or markings; (2) back with small black spots, but without large black markings; (3) back with few black markings, covering ca. 10-15% of the dorsal surface; (4) back with a moderate number of black markings, covering ca. 15-25% of the dorsal surface; (5) back with many large black markings, covering ca. 25-45% of the dorsal surface; (6) back largely covered by black, at least 45% of dorsal surface. Our data refer to a total of 915 specimens (583 males, 84 females, 248 juveniles/subadults). Generally, specimens with more than 50 mm SVL were considered as adults, but a few smaller specimens could also be reliably sexed and were thus not included in the juvenile/subadult category. To understand the correlation of the proportion of black patterning with sex or body size, we analysed the data with the software package SPSS for Windows, version 7.5.

Table 1 shows the occurrence of black patterning separately for males, females, and juveniles/subadults. 38.7% of the males and 17.9% of the females, but none of the juveniles/subadults, had a moderate to large amount of black pattern (classes 4-6). In males, the mean snout-vent length (SVL) of specimens without black pattern (class 1) was smaller, and the mean SVL of specimens with a large amount of black pattern (class 5 and 6) was larger than the overall mean value of 66.5 mm. Clearly, in males, SVL is positively correlated with the amount of black patterning (linear regression analysis; P<0.001). SVL values differed significantly between pattern classes (Kruskal Wallis ANOVA; p<0.001). In females, this correlation was less distinct. Our sample included only a relatively small number of females, and these were rather small (mean SVL 64.9 mm). In Rana temporaria, females are generally larger than males (e.g. Galán 1989; Sperling et al. 1996; Kneitz 1998), and it can therefore be assumed that our sample is not representative for the female population of the Circo de Piedrafita. A slightly significant correlation of black patterning with SVL was found (linear regression analysis; p<0.05), but SVL values did not differ significantly between pattern classes. According to data in Tab. 1, females appeared to have less black patterning than males (Tab 1.); this was confirmed by ANCOVA analysis with SVL as covariate (influence of sex on amount of black pattern with highly significant, p<0.001). However, final conclusions regarding sexual differences of black patterning can only be drawn after a larger and more representative sample of the female population is studied.

 Table 1. Percentages (total number in brackets) of male, female and

 juvenile/subadult R. temporaria specimens showing the different degrees of black

 patterning, and the snout-vent lengths (SVL; mean±standard deviation, range in

 brackets) of the different pattern classes.

Pattern	Males		Females		Juveniles/Subadults	
	Se(N)	SVL (mm)	% (N)	SVL (mm)	% (N)	SVL (mm)
1	20,9(%) (122)	63,99±5.3 (50-80)	40,5% (34)	62,65±9.8 (44-85)	96,8% (240)	22,16±6.3 (15-49)
2	8,7% (51)	66,94±5.8 (52-83)	8,3% (7)	61,57±8.4 (49-72)	1,2% (3)	42,67±6.0 (37-49)
3	31,6% (184)	66,26±5.6 (45-82)	33,3% (28)	667,25±7.4 (55-83)	2,0% (5)	46.00±4.2 (40-50)
4	23,3% (136)	66,48±5.0 (55-81)	14,3% (12)	69,42±5.4 (62-68)	0% (0)	-
5	12,3% (72)	69,40±5.4 (59-84)	1,2% (1)	64,0	0% (0)	1
6	3,1% (18)	71,33±5.5 (62-80)	2,4% (2)	62.50±10.6 (55-70)	0% (0)	

Summarizing, in male and probably also female, *R. temporaria* from Circo de Piedrafita, black dorsal pattern increases with size. SVL in *R. temporaria* is generally correlated with age (e.g. Pintar 1982; Gibbons and McCarthy 1983; Ryser 1986), although this correlation is not a diagnostic one – some adult frogs reaching already in their second year (in lowland habitats) sizes typical for three-year old and older specimens (e.g. Kneitz 1998). We assume that the presence and extension of black pattern is even more correlated with age than with size, as it becomes apparent by the total lack of such spots in one-year old juveniles.

Two mechanisms are a *priori* conceivable as causes of this phenomenon: the extension of black pattern in individual frogs may increase with age; or a differential survival due to selective pressures may account for a higher proportion of black patterned individuals among the larger (older) frogs. The fact that we nearly did not find black markings in the juveniles examined in detail (nor in ca. 2000 additional juveniles examined superficially) clearly supports the first explanation.

In humans, the suntanning effect is well known; melanin production in the deep epidermal layer increases when the skin is exposed to sunlight, and sometimes the



Figure 1. Schematic drawing of the different classes of black pattern as used in this paper.



Plot of Snout-vent lengths (SVL) in male *Rana temporaria* from Circo de Piedrafita grouped in one of the six pattern classes.



Plate 1.

Pyrenean specimen of *R. temporaria* without black spots (class 1) from Aguas Tuertas, Aragon, Spain, photographed in 1997 by M. Vences.



## Plate 2.

Pyrenean specimen of *R. temporaria* with small dispersed black spots (classs 2) from Aguas Tuertas, Aragon, Spain, photographed in 1997 by M. Vences.



Plate 3.

Pyrenean specimen of *R. temporaria* with many large black markings (class 5) from Circo de Piedrafita, Aragon, Spain, photographed in 1998 by M. Vences. Several mutilated specimens (this one lacking the left foreleg) were found in this large population.

melanin is accumulated in irregular patches which externally give rise to freckles. A similar suntanning effect is also known in sharks (Lowe and Goodman-Lowe 1996). Melanin production in humans is stimulated by the MSH hormone (inhibited by MIH) which acts on the skin pigment cells (see e.g. Randall et al. 1997). Currently there are no data to decide whether in *Rana temporaria*, the black pattern is genetically determined (and the corresponding alleles have selective advantages in high mountain habitats), or if the appearance of black pattern is an ontogenetic "suntanning" response to increased ultraviolet radiation or other ecological determinants.

## REFERENCES

- Arnold, E.N., Burton, J.A. and Ovenden, D.W. (1978). A Field Guide to the Reptiles and Amphibians of Britain and Europe. London: Collins.
- Behler, J.L. and King, F.W. (1979). The Audubon Society Field Guide to North American Reptiles and Amphibians. New York: A. Knopf.
- Blaustein, A.R., Hoffman, P.D., Hokit, D.G., Kiesecker, J.M., Walls, S.C. and Hayes, J.B. (1994). UV repair and resistance to solar UV-B in amphibian eggs: a link to population declines? *Proc. Natl. Acad. Sci. USA* 91: 1791-1795.
- Blaustein, A.R. and Wake, D.B. (1995). The Puzzle of Declining Amphibian Populations Scientific American 272 (4): 56-61.
- Galán, P. (1989). Diferenciación morfologica y selección de habitats en las ranas pardas del noroeste iberico: *Rana iberica y Rana temporaria. Treb. Socl. Cat. Ictio. Herp., Barcelona,* 2: 193-209.

- Gibbons, M.M. and McCarthy, T.K. (1983). Age determination of frogs and toads (Amphibia, Anura) from north-western Europe. Zoologica Scripta 12 (2): 145-151.
- Kneitz, S. (1998). Untersuchungen zur Populationsdynamik und zum Ausbreitungsverhalten von Amphibien in der Agrarlandschaft. Bochum: Laurenti Verlag.
- Lowe, C. and Goodman-Lowe, G. (1996). Suntanning in hammerhead sharks. *Nature* 383: 677.
- Nöllert, A. and Nöllert, C. (1992). Die Amphibien Europas. Bestimmung, Gefährdung, Schutz. Stuttgart: Franckh-Kosmos.
- Pedraza, E.M. and Lizana, M. (1997). Primeros datos sobre el efecto de la radiación ultravioleta en el declive de los anfibios españoles. *Quercus* 137: 15-17.
- Pintar, M. (1982). Wachstum von Braufröschen im Frieland (Amphibia: Salientia: Ranidae). Salamandra 18 (3/4): 338-341.
- Randall, D.J., Burggren, W. and French, K. (1997). Eckert Animal Physiology: Mechanisms and Adaptations. 4th edition. New York: W.H. Freeman.
- Ryser, J. (1986). Altersstruktur, Geschlechterverhältnis und Dynamik einer Grasfrosch-Population (*Rana temporaria* L.) aus der Schweiz.-Zool. Anz. 217 (3/4): 234-251.
- Sperling, P., Vences M. and Böhme W. (1996). Vorläufige Bemerkungen zum taxonomischen Status von Rana temporaria honnorati Heron-Royer, 1881. Salamandra 32 (2): 99-112.
- Wake D.B. (1991). Declining amphibian populations. Science 253: 860.