Genet Sel Evol (1991) 23, 263-272

Original article

Polytypic chromosomal variation in *Triturus boscai* (Urodela : Salamandridae)

P Herrero

Unidad de Fisiología Animal, Departamento de Biología, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain

(Received 29 October 1990; accepted 10 May 1991)

Summary – Chromosomal variation in populations of the Iberian endemic species *Triturus boscai* was analyzed by determining the C-heterochromatin distribution and the DNA content. Evidence is presented for the existence of 2 population groups which show sterility in hybrids.

chromosomal differentiation / C-banding patterns / DNA nuclear content / meiotic analysis / *Triturus boscai*

Résumé – Variation polytypique des chromosomes de *Triturus boscai* (Urodèle : Salamandridés). *Nous avons analysé la variation chromosomique de populations de l'espèce endémique ibérique* Triturus boscai *moyennant l'étude de la distribution de l'hétérochromatine et de la quantité d'ADN. Nous présentons des arguments en faveur de l'existence de 2 groupes de populations dont les hybrides montrent une fertilité réduite.*

différenciation chromosomique / distribution des bandes C / ADN nucléaire / analyse méiotique / *Triturus boscai*

INTRODUCTION

Triturus boscai Lataste is an endemic species restricted to the western half of the Iberian Peninsula (García-Paris, 1985; Barbadillo-Escrivá, 1987). Bolkay (1928) included this newt in the small-sized species group of *Triturus* (Paleotriton). However, some other authors have criticized this kinship based on its possible mating with *T alpestris* which belongs to the Mesotriton group (Spurway, 1953; Bucci-Innocenti *et al*, 1983).

The studies on this newt, although very scarce, include some devoted to the analysis of the chromosome complement (Herrero, 1982a,b; Bucci-Innocenti *et al*, 1983). However, there is no information about intraspecific variability in natural populations.

Other species of the genus (*T alpestris* and *T cristatus*) show variation in morphological, chromosomal or molecular characteristics (Thorn, 1968; Kalezic and

Hedgecock, 1980; Bucci-Innocenti *et al*, 1983; Herrero and Arano, 1986; Arano and Arntzen, 1987; Herrero *et al*, 1989; Wallis and Arntzen, 1989).

In this paper we have explored the intraspecific chromosomal variation of *T boscai* according to 2 features (heterochromatin distribution and DNA content) in different populations of the Iberian peninsula. The differences reported in the C-banding pattern of this species are particularly significant for this genus which shows a high degree of chromosome stability.

MATERIAL AND METHODS

The populations studied are summarized in figure 1. We collected $\approx$ 10 males and 10 females from each population, excepting those belonging to Cenicientos, Monfragüe and those of the La Vera region which were sampled during 3 consecutive yr, totaling 30 individuals for each population per year.

Specimens were injected with a 0.3% colchicine solution. After 7 h, they were sacrificed and mitotic chromosomes were prepared directly from both intestine and testes and meiotic chromosomes were obtained from testes. In every case the material was fixed in ethanol : acetic acid (3:1) for at least 24 h.

The C-banding technique applied was that reported by Sumner (1972) with some modifications : mitotic and meiotic chromosomes were incubated in $Ba(OH)_2$ at 60 °C for 15 min and 30 min, respectively.

The C-banding pattern of each individual was obtained after analyzing 5 mitotic metaphases. The chromosomes were paired in the karyotype according to their size and C-banding pattern. Chiasma distribution was determined by observation of 20 diplotene cells for each individual.

Nuclear DNA content of the different individuals was estimated from blood smears stained by Feulgen's reaction. Measurements were taken with a Vickers M-85 integrating microdensitometer at a wavelength of 550 nm. In every case blood smears of *Bufo bufo* were used as a standard. Its DNA content was taken as equal to 100 so that the DNA content of the *T boscai* samples were calculated in relative units. For each individual at least 100 nuclei of erythrocytes were measured to avoid a standard error of the mean higher than 1%. The mean values of DNA content were compared with a Student's *t*-test.

RESULTS

The chromosome complement of *T boscai* consists of 24 chromosomes of decreasing size, where 4 pairs are submetacentric and the remainder are metacentric (Herrero, 1982a).

Heterochromatin distribution

The C-banding patterns of each individual within a given population are identical. However, the patterns show some remarkable differences between distinct populations. One of them is found in the individuals from Madrigal de la Vera, Valverde de la Vera, Villanueva de la Vera and Losar de la Vera (see fig 1). This pattern

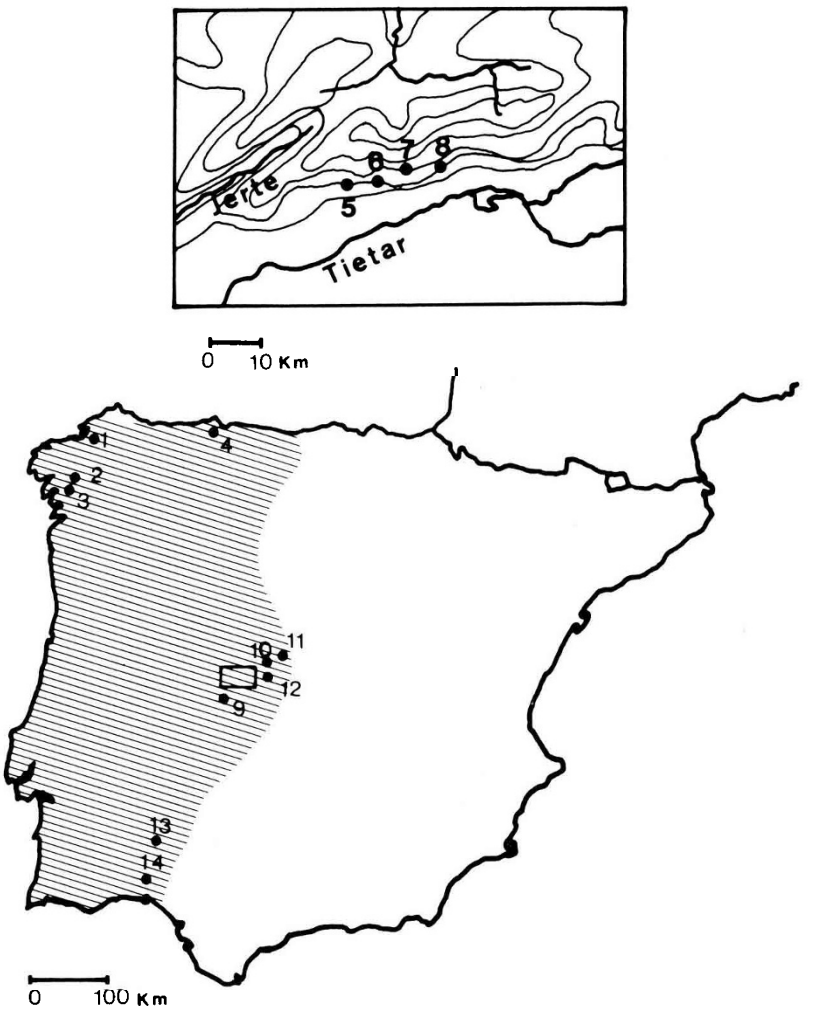

Fig 1. Geographic distribution area (striped zone) and location of the 14 *Triturus boscai* populations sampled in our study. 1, Pontedeume (La Coruña); 2, La Estrada (Pontevedra); 3, Morana (Pontevedra); 4, Oviedo (Asturias); 5, Losar de la Vera (Cáceres); 6, Villanueva de la Vera (Cáceres); 7, Valverde de la Vera (Cáceres); 8, Madrigal de la Vera (Cáceres); 9, Monfragüe (Cáceres); 10, Cenicientos (Madrid); 11, San Martin de Valdeiglesias (Madrid); 12, Pelahustan (Toledo); 13, Gerena (Sevilla); and 14, Almonte (Huelva). Enlarged section shows the 4 sample sites at which polytypic variations was found.

(fig 2a) comprises tiny centromeric bands in all chromosomes excepting pair 11; and 2 pericentric bands on both sides of centromeric regions on all the chromosome pairs excepting pairs 10, 11 and 12. The pericentric bands of pairs 10, 11 and 12 do

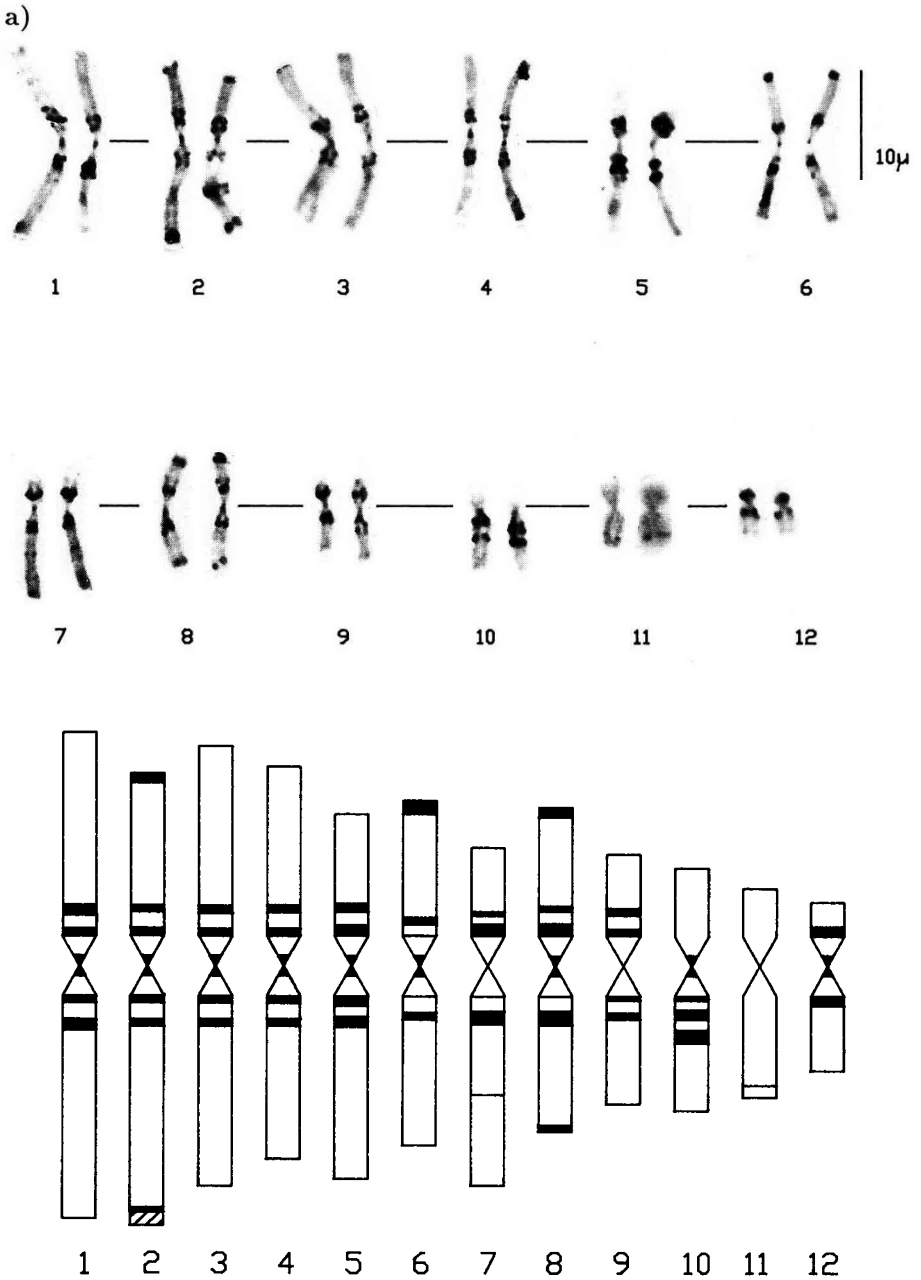

Fig 2. C-banded karyotypes of the 2 population groups of *Triturus boscai* : a), La Vera populations;

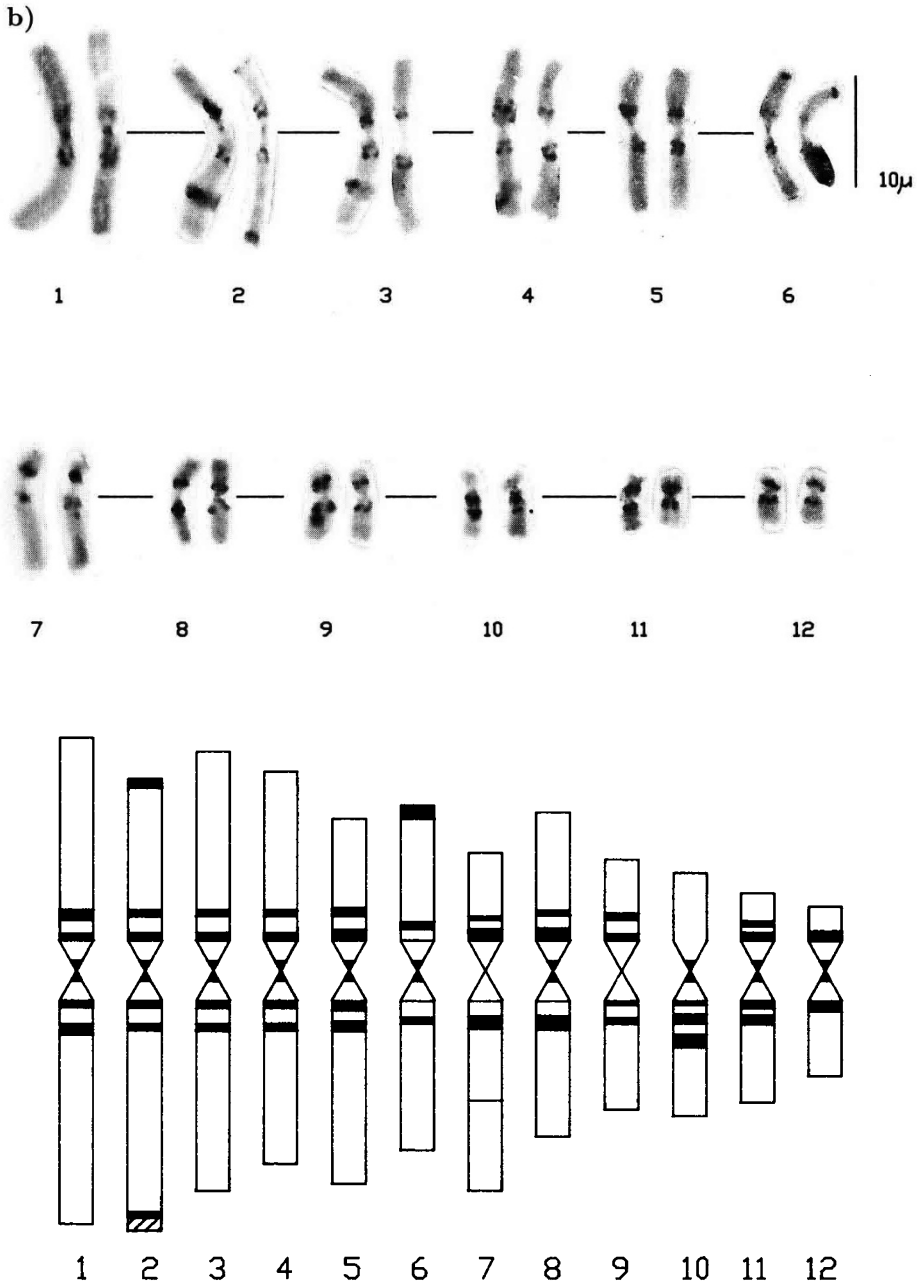

Fig 2. (*suite*) b), Iberian populations excepting La Vera populations.

not have the same distribution. Pair 10 presents a series of thin bands restricted to the long arm that appears as 3 bands when the chromosome is highly condensed; pair 11 has none and pair 12 presents a single one close to the centromeric region (fig 2a). The distance between the pericentric bands located on a given arm varies from 1 chromosome pair to another and sometimes between both arms of the same chromosome. Thus they can be observed as a single band when the chromosomes are highly condensed.

Subterminal bands appear on the long arms of pairs 2 and 11 and on the short arm of pair 8. There are terminal bands on both arms of pair 8 and on the short arm of pair 6. Moreover, pair 7 has an interstitial band on its long arm (fig 2a).

The remaining populations showed differences in the heterochromatin distribution affecting mainly chromosome pairs 8 and 11. In this case, the telomeric bands of pair 8 are not present and pair 11 shows two pericentric bands on both sides of the centromere but lacks the subterminal band on the long arm (fig 2b).

On the other hand, we must emphasize that the centromeric index and relative lengths of these pairs do not show any differences between populations and coincide with those previously described by Herrero (1982a). Moreover, there is a correspondence between the C-banding patterns reported here and those previously reported (Herrero, 1982b; Bucci-Innocenti *et al*, 1983). The pattern corresponding to La Vera populations coincides with that described by Herrero (1982b), where individuals La Vera were also analyzed. The second pattern coincides with that reported by Bucci-Innocenti *et al* (1983) who do not give details on the geographic origin of the specimens studied.

DNA content

The results from DNA cytophotometric measurements are shown in table I. They do not show significant differences between the populations studied. DNA content is almost 4 times higher than that of *Bufo bufo*. The absolute value has been calculated considering 14 pg/N for *B bufo* (Bachmann, 1970).

Table I. DNA amounts of several individuals from different populations of *Triturus boscai.*

Populations	*Relative units*	*pg/Nucleus*
Oviedo	372.52 + 2.00	51.94
Cenicientos	378.93 + 2.90	53.06
Valverde de la Vera	375.70 + 3.61	52.59
Madrigal de la Vera	370.38 + 2.35	51.85
Bufo bufo	100 + 0.42	14

Meiotic behaviour

At diplotene, bivalents form from 1 to 3 chiasmata in terminal, subterminal or interstitial positions (Herrero and López-Fernández, 1986).

However, in one population (Valverde de la Vera) 4 males showed a strong incidence of desynapsis since at diplotene several univalents or bivalents with a single terminal chiasma were always observed (fig 3). These individuals, in spite of the normal size of their gonads, were completely sterile since no further meiotic phases were scored in the preparations that were devoid of any spermatozoa. Interestingly, these individuals may be considered as hybrids between the 2 forms described according to their C-banded pattern since, at least, they were heterozygous for pairs 8 and 11 (fig 3).

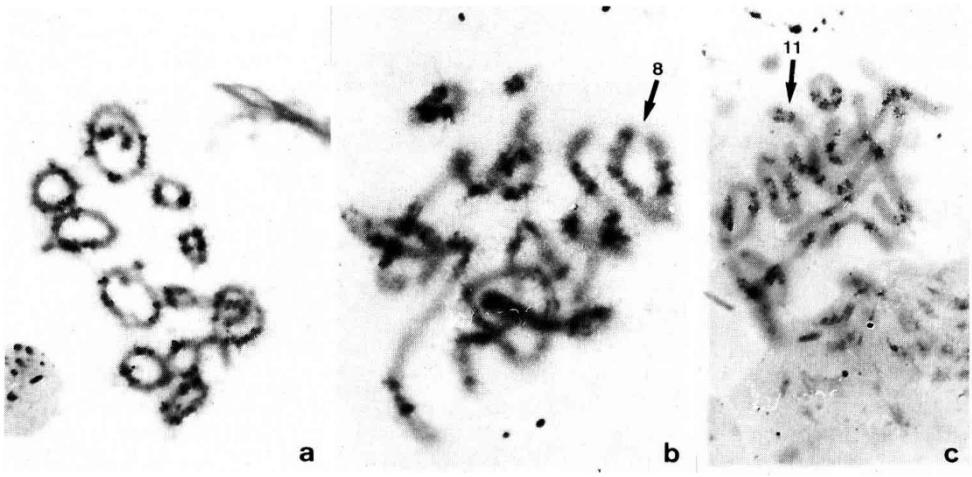

Fig 3. C-banded diplotenes of *Triturus boscai* : a), Spermatocyte of a normal individual; b, c, spermatocytes of hybrids from the Valverde de la Vera population; b), the arrow shows the heterozygous bivalent 8; c), the arrow shows the heterozygous bivalent 11. (Note presence of univalents and bivalents with one terminal chiasma.)

DISCUSSION

Chromosomal divergence

Triturus is a group where no gross chromosome rearrangements have occurred (Mancino *et al*, 1977) except those referred to pair 1 of the *Neotriton* group (Sims *et al*, 1984), or the pericentric inversion described in some populations of *T italicus* (Ragghianti *et al*, 1980). Many authors support the existence of small rearrangements as main events in the chromosome evolution of this genus (Mancino *et al*, 1977; Macgregor *et al*, 1983). However, no clear evidence for this has yet been obtained. For this purpose, studies on intraspecific chromosomal variation in closely related species is desirable, particularly since small rearrangements would be clearly revealed because of the similar characteristics of chromosome constitution in the groups analyzed. However, the chromosomal differentiation found in the *T alpestris* complex (Herrero *et al*, 1989) only refers to the amount of heterochromatin. This also seems to be the case of *T boscai*. The 2 C-banded patterns found cannot be

easily explained by simple rearrangements : the morphology and size of chromosomes are preserved. The differences only refer to 2 pairs (8 and 11) which seem to undergo subtle changes in the amount and distribution of heterochromatin. Accordingly DNA values are not significantly altered, although small DNA variations could not be detected with this method.

King (1980) suggested a euchromatin–heterochromatin transformation process for explaining these phenomena in *Litoria*. However, no further evidence has supported his argument. The alternative model by Macgregor and Sessions (1986) on the growth and dispersion of satellite DNA sequences sequestered in heterochromatin regions of newts of the genus *Triturus* seems more consistent. According to their model, satellite DNA sequences or heterochromatic regions would arise at centromere positions wherefrom they would be dispersed through the genome by successive amplifications and insertions based on unequal sister chromatid exchange, chromosomal rearrangements or some other molecular mechanisms. Although we do not have information on satellite DNA sequences located in the heterochromatic regions of *T boscai*, this model could fit in. However, an important drawback for this hypothesis stems from the short evolutionary time in which the heterochromatin differences in *T boscai* would have occurred, in comparison to the evolutionary times for which the model was proposed.

Heterozygosity and infertility

Whatever the origin of the chromosome differences described, a polytypic variation affecting the Iberian populations of *T boscai* is clearly shown. However, in some areas where populations showing distinct C-band patterns could meet we have found sterile hybrids. The meiotic behaviour of these individuals suggests the existence of mechanisms that prevent normal completion of meiosis. In fact no spermatid nuclei are formed. These results clearly suggest that although hybrids may form and become adults they are sterile. As a consequence, the chromosome differences described may uncover other functions that promote reproductive isolation between both forms.

In summary, Iberian populations of *T boscai* are distributed in at least two groups according to their C-banding patterns : one group is restricted to the La Vera region, located in the Tietar Valley of the Gredos Mountains and the other one extends throughout the remainder of the geographical distribution of this species. Moreover, in places where both groups meet, hybrids present a high chromosomal instability affecting the meiotic behaviour and resulting in a high degree of sterility.

ACKNOWLEDGMENTS

I appreciate very much the helpful comments and suggestions of Dr G de la Vega. I would also like to thank Dr Navarrete for allowing me the use of the Vickers M-85 Integrating Microdensitometer, and J García Herranz and J Dorda Dorda for their technical assistance. The specimens have been collected with official permission obtained from regional authorities. This work was supported by DGICYT PB 880010 Project (Spain) and by a Cooperative Project (Spain–Great Britain) HB-180.

REFERENCES

Arano B, Arntzen JW (1987) Genetic differentiation in the alpine newt *Triturus alpestris. In : Proceedings, 4th Ordinary General Meeting SEH, Nijmegen 1987* (Van Gelder JJ, Strijbosch H, Bergers PJM, eds) Fac Sci, Nijmegen Press, Nijmegen, The Netherlands, 21-24

Bachmann K (1970) Specific nuclear DNA amounts in toads of the genus *Bufo. Chromosoma* 29, 365-379

Barbadillo-Escrivá LJ (1987) *La Guía de INCAFO de los Anfibios y Reptiles de la Península Ibérica, Islas Baleares y Canarias.* Incafo SA, Madrid

Bolkay STJ (1928) Die Schadel der Salamandrinen, mit besonderer Rucksicht auf ihre systematische Bedeutung. *Zwitsch Anat Entwicklungsgesch* 86, 259-319

Bucci-Innocenti S, Ragghianti M, Mancino G (1983) Investigations of karyology and hybrids in *Triturus boscai* and *T vittatus*, with a reinterpretation of the species groups within *Triturus* (Caudata : Salamandridae). *Copeia* 1983, 662-672

García-Paris M (1985) *Los Anfibios de España.* Publicaciones de Extensión Agraria, Madrid

Herrero P (1982a) Karyotypes of two Iberian amphibians : *Rana iberica* and *Triturus boscai. Herpetologica* 38, 502-506

Herrero P (1982b) Heterochromatin distribution in the chromosome complement of *Triturus boscai* (Caudata : Salamandridae). *Amphibia-Reptilia* 4, 309-315

Herrero P, Arano B (1986) Cytogenetic and morphological studies on *Triturus alpestris cyreni. In : Studies in Herpetology* (Rocek Z, ed) Charles University Press, Prague, 151-154

Herrero P, López-Fernández C (1986) The meiotic system of Iberian species of the genus *Triturus* (Amphibia : Caudata). *Caryologia* 39, 385-395

Herrero P, Arano B, García de la Vega C (1989) Chromosome differentiation in the *Triturus alpestris* complex (Amphibia : Caudata). *Genetica* 79, 27-35

Kalezic ML, Hedgecock D (1980) Genetic variation and differentiation of three common European newts (*Triturus*) in Yugoslavia. *Br J Herpetol* 6, 49-57

King M (1980) C-banding studies on Australian Hylid frogs : secondary constriction structure and the concept of euchromatin transformation. *Chromosoma* 80, 191-217

Macgregor HC, Horner HA, Sims SH (1983) Newt chromosomes and some problems in evolutionary cytogenetics. *In : Kew Chromosome Conference II* (Brandhom PH, Bennett MD, eds) Allen & Unwin Ltd, 283-294

Macgregor HC, Sessions SK (1986) The biological significance of variation in satellite DNA and heterochromatin in newts of the genus *Triturus* : an evolutionary perspective. *Phil Trans R Soc Lond* B312, 243-259

Mancino G, Ragghianti M, Bucci-Innocenti S (1977) Cytotaxonomy and cytogenetics in European newt species. *In : The Reproductive Biology of Amphibians* (Taylor DH, Guttman SF, eds) Plenum Press, NY, 411-447

Ragghianti M, Bucci-Innocenti S, Mancino G (1980) Chromosome polymorphism in the Italian newt. *Triturus italicus. Chromosoma* 77, 333-345

Sims SH, Macgregor HC, Pellat PS, Horner HA (1984) Chromosome I in crested and marbled newts (*Triturus*). An extraordinary case of heteromorphism and independent chromosome evolution. *Chromosoma* 89, 169-185

Spurway H (1953) Genetics of specific and subspecific differences in European newts. *Symp Soc Exp Biol* 7, 200-237

Sumner AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res* 75, 304-306

Thorn R (1968) *Les Salamandres d'Europe, d'Asie et d'Afrique du Nord.* Lechevalier, Paris

Wallis GP, Arntzen JW (1989) Mitochondrial-DNA variation in the crested newt superspecies : limited cytoplasmic gene flow among species. *Evolution* 43, 88-104