

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*

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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; Kalezić 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 ≈ 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 $\text{Ba}(\text{OH})_2$ 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

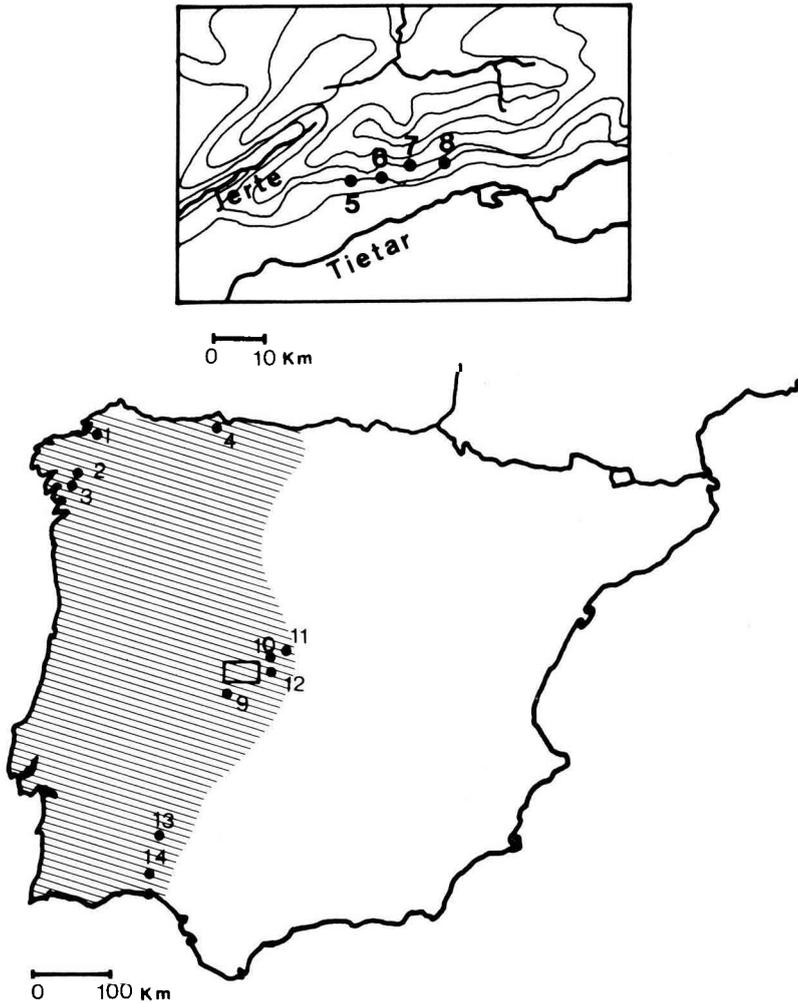


Fig 1. Geographic distribution area (striped zone) and location of the 14 *Triturus boscai* populations sampled in our study. 1, Pontedeume (La Coruña); 2, La Estrada (Pontevedra); 3, Morana (Pontevedra); 4, Oviedo (Asturias); 5, Losar de la Vera (Cáceres); 6, Villanueva de la Vera (Cáceres); 7, Valverde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

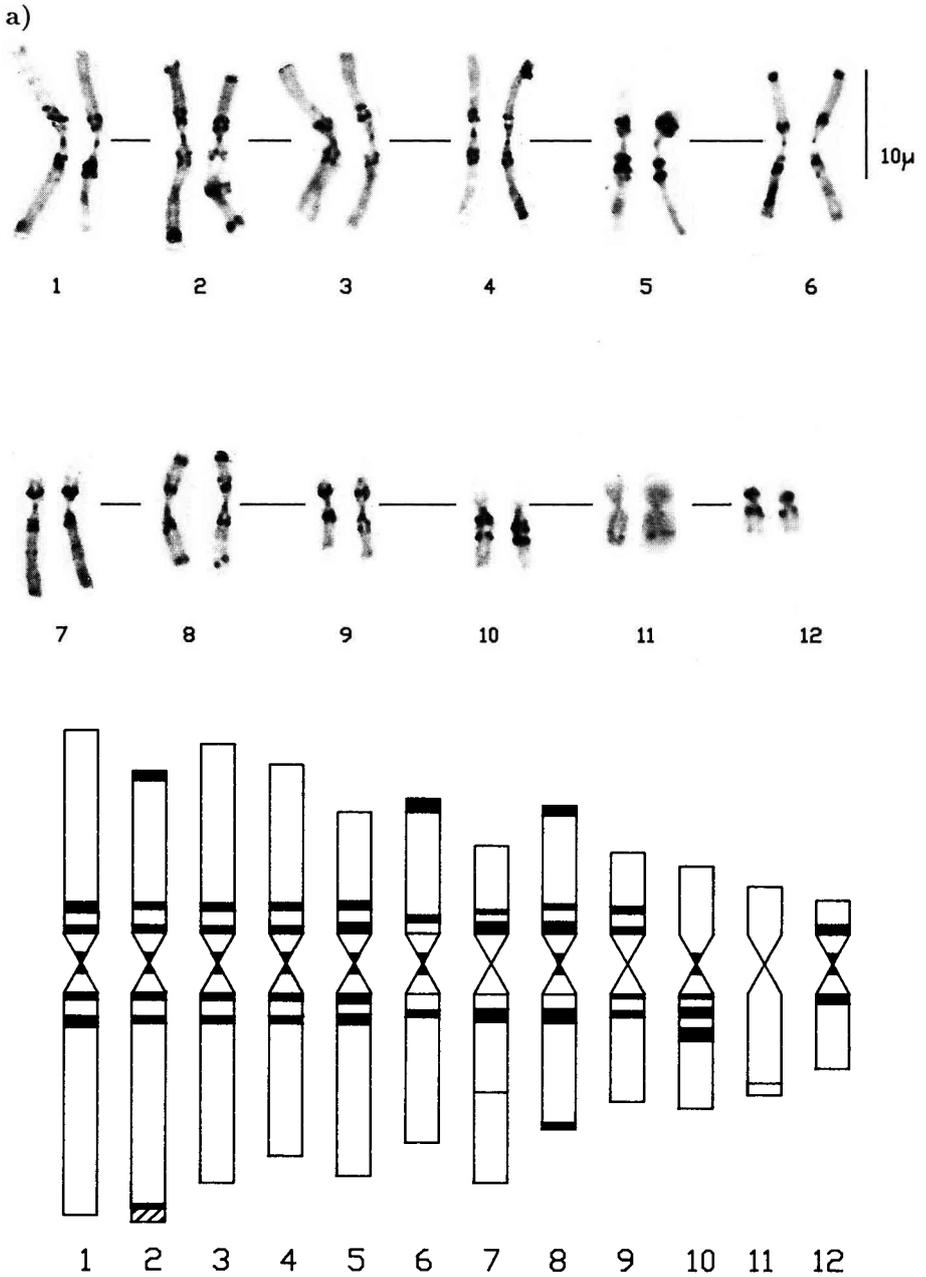


Fig 2. C-banded karyotypes of the 2 population groups of *Triturus boscai* : a), La Vera populations;

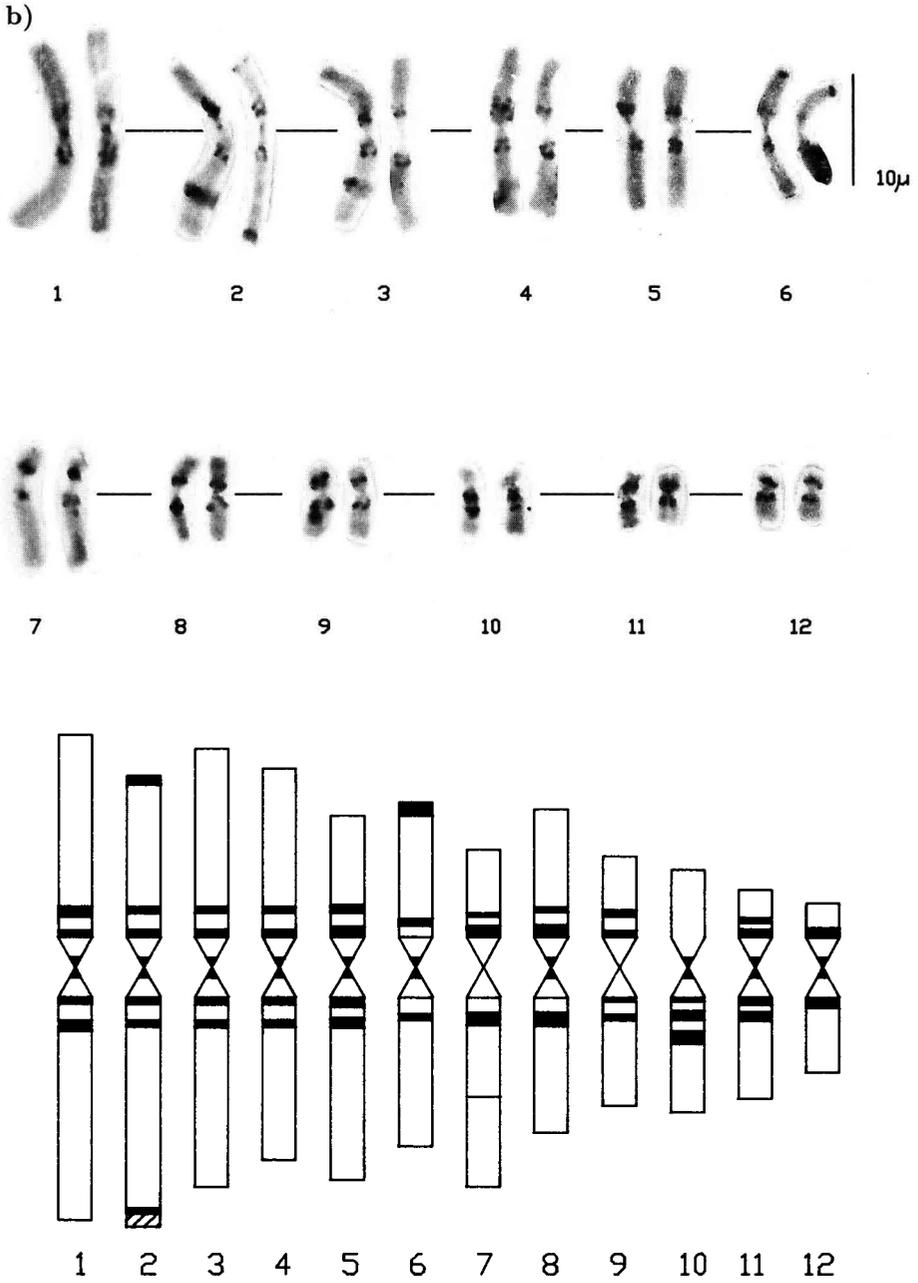


Fig 2. (suite) b), Iberian populations excepting La Vera populations.

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*.

<i>Populations</i>	<i>Relative units</i>	<i>pg/Nucleus</i>
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
<i>Bufo bufo</i>	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).

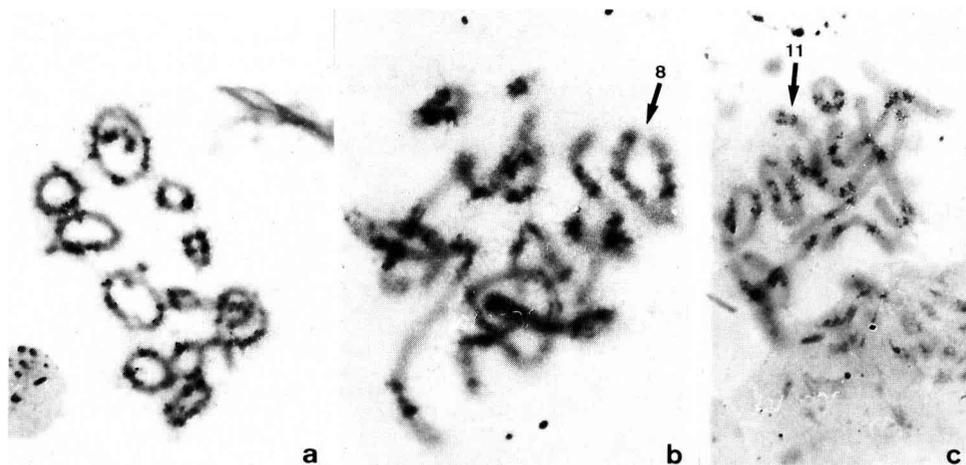


Fig 3. C-banded diplotenes of *Triturus boscai*: a), Spermatocyte of a normal individual; b, c, spermatocytes of hybrids from the Valverde de la Vera population; b), the arrow shows the heterozygous bivalent 8; c), the arrow shows the heterozygous bivalent 11. (Note presence of univalents and bivalents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

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