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The karyotype of *Lacerta horváthi* (Reptilia, Sauria, Lacertidae)

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Abstract

The chromosomes of *Lacerta horváthi* have been studied by means of conventional, C-banding, and silver-NOR techniques. The karyotype of this species, characterized by 36 acrocentric macrochromosomes, lacks the typical pair of microchromosomes shared by all other lacertid lizards. It is hypothesized that the microchromosomes could have been translocated to the large elements of the karyotype. The occurrence of such a rearrangement in the chromosome complement of *L. horváthi* underlines its isolation from the other species of the subgenus *Archaeolacerta*. The C-banding analysis evidences the existence of a female sex heteromorphism in which the W-chromosome has the same shape and size of the Z, but differs from it in being completely heterochromatic. The nucleolar organizer regions (NORs) are located on a pair of medium size chromosomes in subtelomeric position, where the standard Giemsa-staining reveals secondary constrictions.

Introduction

Lacerta horváthi Méhely, 1904 is a rather unknown lacertid lizard present, with scattered populations, in northern and central Jugoslavia (Slovenia, Istria, Dalmatia), in south-western Austria (Carinthia), and in north-eastern Italy (Carnic and Julian Alps) (Lapini & Dolce, 1983; Grillitsch & Tiedemann, 1986).

This species, included by Arnold (1973) in the polymorphic grouping *Lacerta* part II, is assigned to a problematic group of lacertid lizards (*Archaeolacerta*) considered either as a distinct genus (Lanza *et al.*, 1977; Guillaume & Lanza, 1982), or as a subgenus (Mayer & Tiedemann, 1982; Lutz & Mayer, 1985; Lutz *et al.*, 1986). Concerning the phylogenetic relationships, *L. horváthi* seems to be closely related to *L. bedriagae* and *L. oxycephala*, as pointed out by Mayer and Tiedemann (1982) and Lutz and Mayer (1985) on the basis of protein electrophoresis and microcomplement fixation analysis.

Karyological data are available for the latter two species (Gorman *et al.*, 1970; Capula *et al.*, 1982) as well as for 17 other members of the same group (see Table 1), but the karyotype of *L. horváthi* has not yet been described. Consequently we considered interesting to analyse the karyology of this species in order to point out its possible cytotaxonomic relationships within the subgenus *Archaeolacerta*.

Material and methods

Six males and 4 females were utilized for the karyological analysis. They were collected in the following localities:

a. Pierabec, 1100 m a.s.l. (Forni Avoltri, Udine, NE Italy)

Table 1. Current data on karyology of lacertid lizard of the subgenus *Archaeolacerta*.

Species	2n	Karyotype	References
L. armeniaca (p)	38	36MI, 2m	Kupriyanova, 1969
L. bedriagae	38	36MI, 2m	Capula *et al.*, 1982
L. caucasica	38	36MI, 2m	Kupriyanova, 1976
L. dahli (p)	38	36MI, 2m	Kupriyanova, 1969
L. derjugini	38	36MI, 2m	Orlova & Orlov, 1969
L. graeca	38	36MI, 2m	Olmo *et al.*, 1987b
L. horváthi	36	36MI	This paper
L. laevis	38	36MI, 2m	Gorman, 1969
L. mixta	36	32MI, 2MV, 2m	Darevsky & Kulikova, 1961
L. monticola	38	36MI, 2m	Olmo *et al.*, 1987b
L. oxycephala	38	36MI, 2m	Gorman *et al.*, 1970
L. parva	24	14MV, 10MI	Gorman, 1969
L. parvula	38	36MI, 2m	Kupriyanova, 1976
L. portschinskii	38	36MI, 2m	Darevsky & Kupriyanova, 1982
L. raddei	36	32MI, 2MV, 2m	Darevsky & Kulikova, 1961
L. rostombekovi (p)	38	36MI, 2m	Kupriyanova, 1981
L. rudis	36	32MI, 2MV, 2m	Darevsky & Kulikova, 1961
L. saxicola	38	36MI, 2m	Kupriyanova, 1969
L. unisexualis (p)	38	36MI, 2m	Kupriyanova, 1969
L. valentini	36	32MI, 2MV, 2m	Darevsky & Kulikova, 1961

(p) = parthenogenetic species; M = macrochromosomes; m = microchromosomes; V = metacentrics; I = acrocentrics.

b. Rio Bianco Valley, 700 m a.s.l. (Resia, Udine, NE Italy)
c. Jasenak, 900 m a.s.l. (Ogulin, Croatia, central Jugoslavia)

The animals were injected intraperitoneally with Vinblastine sulphate (Velban)® at a concentration of 0.25 mg/ml (0.01 ml for each 2 g of body weight) and, 1 h later, anesthesized with ethyl ether and then dissected. Somatic metaphases were evidenced in bone marrow cells by using standard air-drying techniques.

Meiotic preparations were obtained from testes according to the techniques of Evans *et al.* (1964). The hypotonic solution used was 0.075 M KCl and the fixative was a solution of 3:1 methanol: glacial acetic acid. C-banding preparations were performed according to the Sumner's (1972) method, partially modified as reported by Olmo *et al.* (1984). The slides were stained with 2% Giemsa in 0.1 M phosphate buffer at pH 7, for 10'. The NOR was identified by the silver-staining method of Howell and Black (1980).

Results and discussion

All the examined specimens of *L. horváthi* are characterized by 36 acrocentric macrochromosomes (2n = 36; Fundamental Number (N.F.) = 36), since the typical pair of microchromosome of the lacertid karyotype lacks completely (Fig. 1a). Eighteen bivalents, revealed by the analysis of the male meiotic metaphases (Fig. 2), support this evidence. On some preparations where the chromosomes are little shortened by the action of the cytostatics, a secondary constriction is clearly evidenced in a pair of medium sized chromosomes (pair no. 7) (Figs. 1a, 3).

Among Lacertidae the only other species in which the typical pair of microchromosomes is lacking is *L. vivipara* (2n = 36; N.F. = 36) (Margot, 1946; Chevalier, 1969; Chevalier *et al.*, 1979), a lizard assigned to the subgenus *Zootoca* (Arnold, 1973). The absence of microchromosomes in the karyotype of both *L. horváthi* and *L. vivipara*, opens a phylogenetic controversy: on

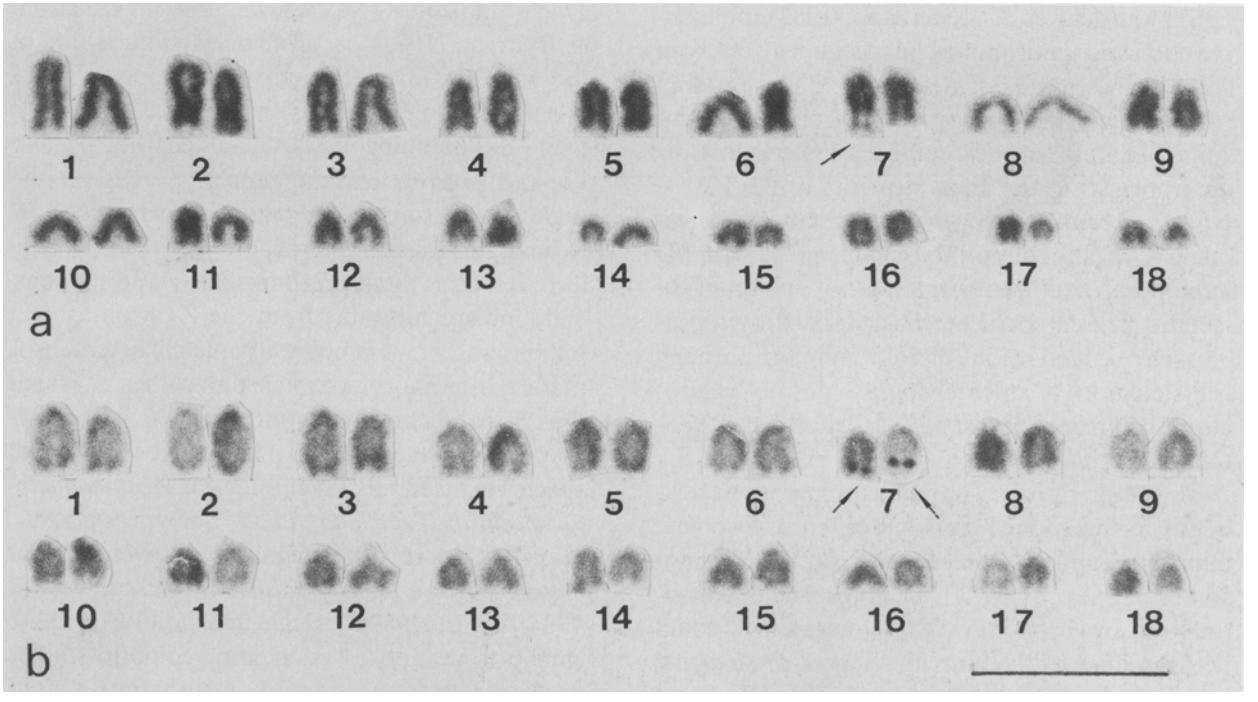

Fig. 1. Lacerta horváthi; (a) conventional- and (b) silver-stained karyotype. The arrow in (a) points to the secondary constriction; the arrows in (b) indicate NOR-localization on the chromosome pair no. 7. Bar, 10 μm.

one hand since these species share the same fundamental number and present a similar structure of the spiny epithelium of the hemipenis, a 'constant, atelic character with high taxonomic value' according to Böhme (1971), it would seem that they belong to the same species group, as proposed by Böhme (1971); on the other since *L. horváthi* and *L. vivipara* are genetically well differentiated (Nei's genetic distance (*D*) = 1.15, ac-

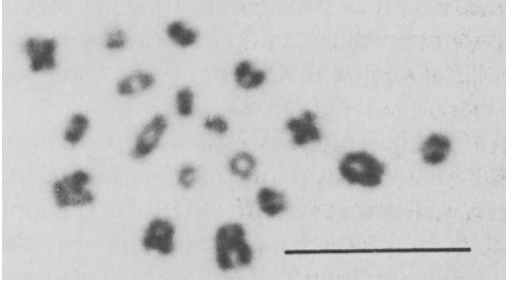

Fig. 2. Lacerta horváthi; male meiotic metaphase. Bar, 10 μm.

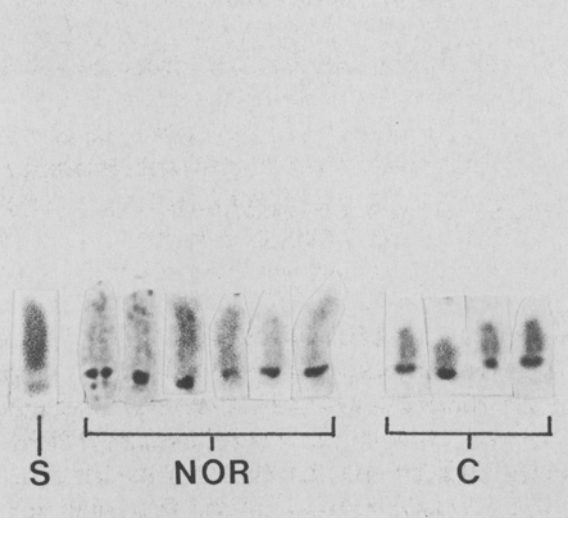

Fig. 3. Lacerta horváthi; (S) conventional, (NOR) silver-NOR, and (C) C-band stainings of the chromosome pair no. 7 from different metaphases. Note that the silver dots are localized in subtelomeric position and correspond to the secondary constriction, while the C-bands occupy the whole telomeric segment, including the secondary constriction.

cording to Mayer & Tiedemann, 1982) the cytogenetic and morphological affinities between these lizards could be interpreted as a by-product of evolutionary convergence. The microchromosomes of both species could have been lost, or incorporated in the large elements of the karyotype as a result of a non-Robertsonian rearrangement. The latter supposition well agrees with the hypothesis that the karyological evolution of lacertid lizards could be characterized by a progressive reduction in diploid number through translocation of microchromosomes to macrochromosomes (Cobror, 1985; Olmo *et al.*, 1986; Odierna *et al.*, 1987).

The analysis of C-banding preparations evidences centromeric bands on few chromosomes; telomeric bands are present only on the chromosomes pair no. 7 (Fig. 4). The presence of constitutive heterochromatin at the telomeric level could be due either to the amplification of pre-existing heterochromatic blocks (Nagl, 1978) or – as reported above – to the translocation of completely heterochromatic microchromosomes to the macrochromosomes (Stock *et al.*, 1974; Tegelström & Ryttman, 1981). In all females studied one of the smallest macrochromosomes appears completely and intensely stained (C-band positive), while the homologous is unstained (Fig. 4). No C-band positive chromosome is present in the male chromosome complement. This result clearly indicates female heterogamety of the ZW type in *L. horváthi*: the W-chromosome is morphologically indistinguishable from the Z-chromosome, but differs from it in being completely heterochromatic. Homomorphic sex chromosomes, in which the Z is euchromatic and the W is heterochromatic, have been so far described in some species of the family Lacertidae, i.e. *Acanthodactylus erythrurus, Gallotia galloti, Lacerta lepida, L. monticola, Meroles cuneirostris, Psammodromus algirus, Takydromus sexlineatus* (Olmo *et al.*, 1984; 1986; 1987a). Such a condition is quite similar to that observed in some colubrid snakes and may represent a primitive state in the differentiation of sex chromosomes (Singh *et al.*, 1976; 1980; Olmo *et al.*, 1987a).

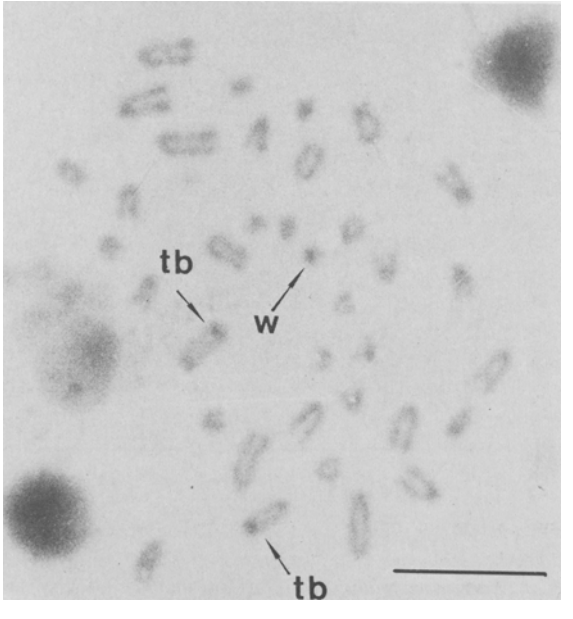

Fig. 4. Lacerta horváthi; female C-banded metaphase. Note the completely heterochromatic W-chromosome (W arrow) and the chromosome pair with telomeric bands (tb arrows). Bar, 10 μm.

The nucleolar organizer of *L. horváthi* is located on the chromosome pair no. 7 (a medium sized pair of macrochromosomes) and occupies a subtelomeric position, corresponding to the location of the secondary constriction evidenced in the conventionally stained preparations (Figs. 1a, 1b, 3, 5). In the other lacertid lizards of the subgenus *Archaeolacerta* for which data on the nucleolar organizer position are available, i.e. *L. graeca* and *L. monticola*, the situation is heterogeneous. In *L. graeca* the NOR is located on chromosomes that are similar in size to the NOR-bearing ones of the species of the genus *Podarcis*, while in *L. monticola* it is situated on a pair of medium-small chromosomes and occupies a subterminal position (Odierna & Cobror, 1986; Olmo *et al.*, 1987b).

Among the species assigned to the subgenus *Archaeolacerta* only *L. mixta, L. raddei, L. rudis*, and *L. valentini* are characterized by the diploid number 2n = 36 (Darevsky & Kulikova, 1961), but, differently from *L. horváthi*, in these lacertid lizards the typical pair of microchromosomes is always present and the fundamental number is 38,

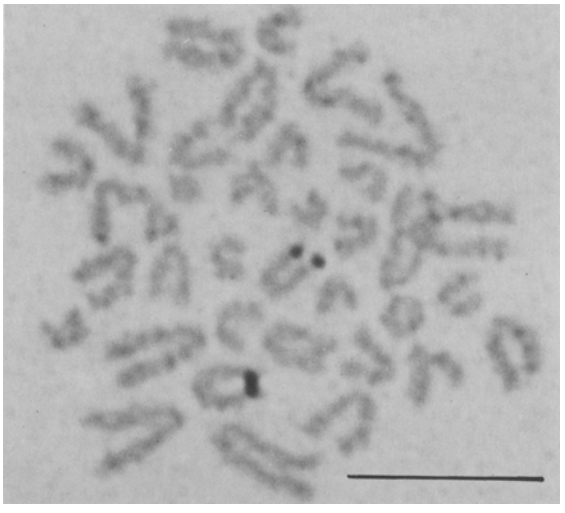

Fig. 5.. Lacerta horváthi; silver stained metaphasic plate. Bar, 10 μm.

the 2 metacentric macrochromosomes of their karyotype being derived by centric fusions. The fundamental number is again 38 in *L. parva*, a species in which the diploid number is highly reduced (2n = 24). This species possesses an unusual karyotype, with 14 metacentric and 10 acrocentric chromosomes, which results from a process of multiple centric fusions (Gorman, 1973; Kupriyanova, 1980).

Since (*i*) *L. horváthi* is the only member of the *Archaeolacerta* group in which microchromosomes lack completely, and (*ii*) since the hypothesized incorporation of microchromosomes in the macrochromosomes probably results in this species from a non-Robertsonian rearrangement rarely occurring in the family Lacertidae, we may suppose that the non-standard karyotype of *L. horváthi* testifies to its isolation from the other species of the subgenus *Archaeolacerta*.

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