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Zoologia. — *The karyotype of* Lacerta mosorensis *(Reptilia: Lacertidae): evidence for a new case of female heterogamety in a lacertid lizard.* Nota di MASSIMO CAPULA e LUCA LAPINI, presentata (*) dal Corrisp. E. CAPANNA.

ABSTRACT. — The chromosome complement of *Lacerta mosorensis* was analyzed by means of conventional techniques in both somatic and spermatogonial metaphases. This species exhibits a typical lacertid karyotype, being characterized by the diploid number 2n = 38 (Fundamental Number = 38). In males the analysis of somatic metaphases reveals 36 acrocentric macrochromosomes and 2 microchromosomes (36 MI + 2m). In females all the examined somatic metaphases are characterized by 35 acrocentric macrochromosomes and 3 small dot-shaped microchromosomes (35 MI + 3 m). This result clearly indicates that in *Lacerta mosorensis* the female is heterogametic. The sex-determining mechanism is of the ZZ/ZW type; in the female karyotype the Z-chromosome is a large sized macrochromosome, while the W-chromosome is equal in size to the two micro-autosomes.

KEY WORDS: *Lacerta mosorensis*; Lacertidae; Karyotype; Female heterogamety.

RIASSUNTO. — *Il cariotipo di* Lacerta mosorensis: *un nuovo caso di eterogametia femminile in una specie della famiglia Lacertidae (Reptilia).* Il cariotipo di *Lacerta mosorensis* è stato studiato utilizzando tecniche convenzionali di preparazione dei cromosomi da midollo osseo e testicoli. Il corredo cromosomico di questa specie è quello caratteristico dei Lacertidi: *L. mosorensis* è infatti caratterizzata da numero diploide 2n = 38 (Numero Fondamentale = 38). Nei maschi l'analisi delle metafasi somatiche rivela la presenza di 36 macrocromosomi acrocentrici e 2 microcromosomi (36 MI + 2m). Le metafasi somatiche delle femmine sono invece caratterizzate costantemente da 35 macrocromosomi acrocentrici e 3 microcromosomi (35 MI + 3 m). Ciò indica chiaramente che in *Lacerta mosorensis* esiste eterogametia femminile. Il meccanismo di determinazione del sesso è del tipo ZZ/ZW. Il cromosoma Z del cariotipo femminile è un macrocromosoma di grandi dimensioni, mentre il cromosoma W è piuttosto piccolo ed ha dimensioni simili a quelle dei due micro-autosomi.

INTRODUCTION

The Mosor rock lizard (*Lacerta mosorensis* Kolombatović, 1886) is a relatively poorly known species endemic to southwestern Jugoslavia, where it inhabits the mountain regions of Dalmatia, Herzegovina and Montenegro (Arnold *et al.*, 1978; Bischoff, 1984*b*). This species is assigned to a problematic group of lacertid lizards, *i.e* *Archaeolacerta*, considered either as a distinct genus (Lanza *et al.*, 1977; Guillaume and Lanza, 1982), or as a subgenus of *Lacerta* (Mayer and Tiedemann, 1982; Lutz and Mayer, 1985; Lutz *et al.*, 1986). According to Méhely (1904, 1910) and Arnold (1973), which studied the osteological characters, the external morphology and colouring of the species included in the subgenus *Archaeolacerta, L. mosorensis* would be closely related to *L. horvathi* Méhely 1904, a rock lizard occurring in north-western Jugoslavia, southwestern Austria, northeastern Italy and southern F. R. Germany (Lapini and Dolce, 1983; Bischoff, 1984*a*; Capula and Luiselli, in press).

(*) Nella seduta del 14 giugno 1990.

Although karyological data are available for *L. horvathi* (De Luca and Dulić, 1988; Capula *et al.*, 1989) as well as for 19 other species of the subgenus *Archaeolacerta* (see Capula *et al.*, 1989), the karyotype of *L. mosorensis* has not yet been described. In order to point out the genetic relationships among the European species of the *Archaeolacerta* group by means of both multilocus electrophoresis (Capula *et al.*, data unpublished, manuscript in preparation) and karyotype analysis (Capula *et al.*, 1982; 1989), we considered interesting to analyze the chromosome complement of the Mosor rock lizard.

Material and methods

Two adult males and two adult females were utilized for the karyological analysis. They were collected in the surroundings of Boračko Jezero, 1100 m a.s.l., (Mostar, Prenj Mountains, SW Jugoslavia), in August 1988.

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 technique 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. The slides were stained by 4% Giemsa in 0.1 M phosphate buffer at pH 7.0 for 5-10′.

A total of 42 somatic metaphases and 35 spermatogonial metaphases (20 diakinesis and 15 metaphases II) were examined.

Results and discussion

All the examined specimens of *Lacerta mosorensis* exhibit a typical lacertid karyotype (*sensu* Gorman, 1973), being characterized by the diploid number 2n = 38 (Fundamental Number (N.F.) = 38).

In males the analysis of the somatic metaphases from bone marrow cells reveals 36 acrocentric macrochromosomes and 2 microchromosomes (figs. 1*a*, 2*a*). All the macrochromosomes gradually decrease in size, so they cannot be divided into separate groups. The spermatogonial metaphases also reveal the 36 MI + 2 m condition (*sensu* Gorman, 1973), and in diakinesis 19 bivalents are clearly evident (fig. 3). The bivalent formed by the pair of microchromosomes is easily distinguishable from the other bivalents because of its small size.

Females are characterized by a different chromosome set: in all the examined somatic metaphases 35 acrocentric macrochromosomes and 3 small dot-shaped microchromosomes (35 MI + 3m) are evident (figs. 1*b*, 2*b*). This result clearly indicates that in *Lacerta mosorensis* the female is heterogametic. The sex determining mechanism is of the ZZ/ZW type. The Z-chromosome is a large sized macrochromosome, while the W-chromosome is heteromorphic and equal in size to the micro-autosomes (fig. 1*b*).

Morphologically differentiated sex chromosomes in which the Z is a macrochro-

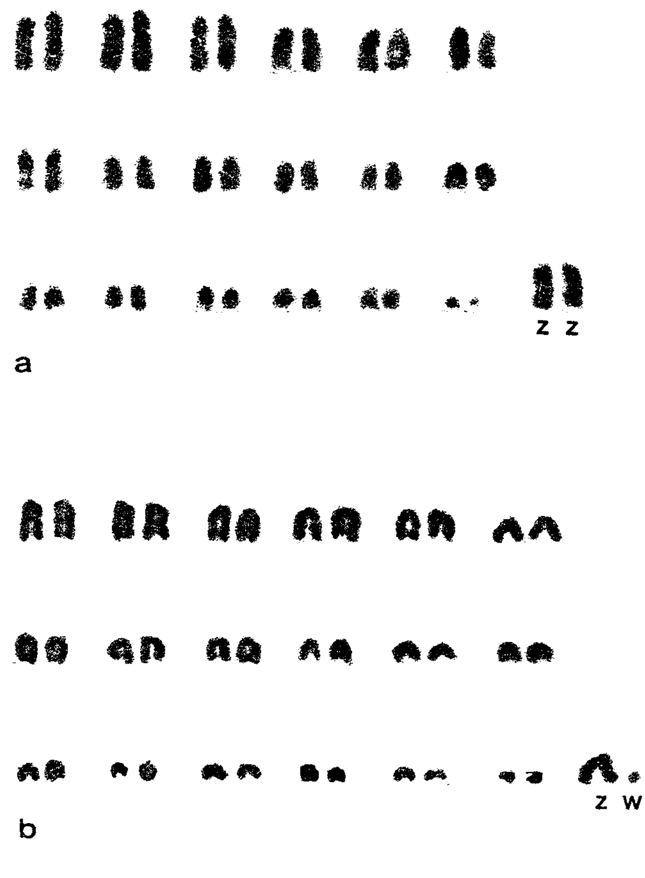

Fig. 1. – Karyotype of *Lacerta mosorensis*: *a*) male; *b*) female (Bar: 10 μm).

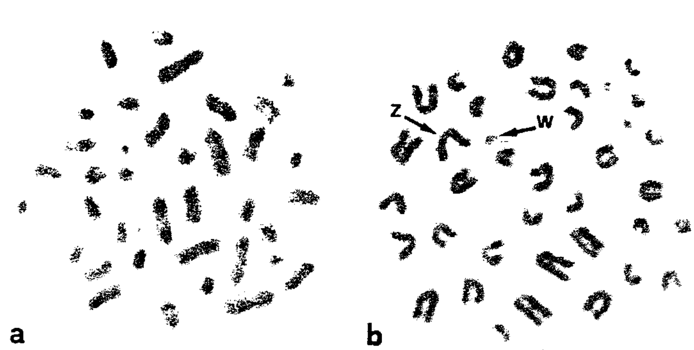

Fig. 2. – *Lacerta mosorensis*: somatic metaphases from bone marrow cells: *a*) male; *b*) female. The arrows in (*b*) indicate the pair of heteromorphic sex chromosomes (Bar: 10 μm).

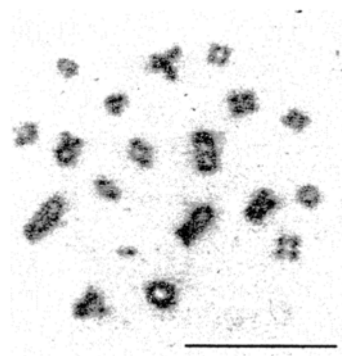

Fig. 3. – *Lacerta mosorensis*: male meiotic diakinesis (Bar: 10 μm).

mosome and the W is a microchromosome – which presumably derived by partial deletion of a macrochromosome (Olmo, 1986) – have been so far reported in 13 species of lacertid lizards (see table I), although some of the described reports seem to be rather doubtful. In all species studied in which sex chromosomes are heteromorphic, *i.e.* distinguishable by their morphology, the Z-chromosome is one of the smallest macrochromosomes or it is equal in size to the last pair of macro-autosomes (Gorman, 1969; Ivanov and Fedorova, 1970; Bhatnagar and Yoniss, 1976; De Smet, 1981; Darevsky and Kupriyanova, 1982; Olmo *et al.*, 1984). On the contrary, as reported above, the Z of the female karyotype of *Lacerta mosorensis* is a large macrochromosome, being comparable in size to the no. 4 chromosome pair (fig. 2*b*).

TABLE I.– *Lacertid lizards in which heteromorphic sex-chromosomes have been described (female heterogamety of the ZZ/ZW type).*

Species	2n	Female	Morphology of sex-chromosomes	References
Eremias arguta	38	35 MI + 3m	Z(Ms), W(m)	Ivanov and Fedorova, 1973
Eremias olivieri	38	35 MI + 3m	Z(Ms), W(m)	Gorman, 1969
Eremias velox	38	35 MI + 3m	Z(Ms), W(m)	Ivanov *et al.*, 1973
Lacerta agilis	38	35 MI + 3m	Z(Ms), W(m)	De Smet, 1981
Lacerta dugesii	38	35 MI + 3m	Z(Ms), W(m) hc	Olmo *et al.*, 1987
Lacerta lepida	36	2 MV, 31 MI + 3m	Z(Ms), W(m)	Olmo *et al.*, 1986
Lacerta mosorensis	38	35 MI + 3m	Z(Ml), W(m)	This paper
Lacerta strigata	38	35 MI + 3m	Z(Ms), W(m)	Ivanov and Fedorova, 1970
Lacerta trilineata	38	35 MI + 3m	Z(Ms), W(m)	Gorman, 1969
Lacerta viridis	38	35 MI + 3m	Z(Ms), W(Mm) hc	De Smet, 1981; Olmo *et al.*, 1985
Ophisops elegans	38	35 MI + 3m	Z(Ms), W(m)	Bhatnagar and Yoniss, 1976
Podarcis melisellensis	38	35 MI + 3m	Z(Ms), W(m)	De Smet, 1981
Podarcis sicula	38	35 MI + 3m	Z(Ms), W(m)	De Smet, 1981
Psammodromus algirus	40	37 MI + 3m	Z(Ms), W(m)	De Smet, 1981

M = macrochromosomes; m = microchromosomes; I = acrocentrics; V = metacentrics.
Sex-chromosomes morphology: Z(Ms) = Z comparable in size to the last pair of macro-autosomes; Z(Ml) = Z comparable in size to a large pair of macro-autosomes; W(m) = W comparable in size to a microchromosome; W(Mm) = W intermediate in size between the last pair of macro-autosomes and the pair of microchromosomes; hc = W heterochromatic.

The karyotype morphology of *Lacerta mosorensis* is typical within the Lacertidae, but greatly differs from that described for the presumed closely related *L. horvathi*. In the latter species the typical pair of microchromosomes of the lacertid karyotype lacks completely and the diploid number is 2n = 36 (N.F. = 36) (De Luca and Dulić, 1988; Capula *et al.*, 1989). Moreover, *L. horvathi* is characterized by a sex-chromosome system different from that observed in *L. mosorensis*: in the female karyotype of the Horvath's rock lizard sex chromosomes are homomorphic, and the W differs from the Z only in being completely heterochromatic and C-band positive (Capula *et al.*, 1989). Such a condition presumably represents a simpler and more primitive sex-determining mechanism than that in which sex chromosomes are heteromorphic (Singh *et al.*, 1976; 1980; Olmo *et al.*, 1987).

Both a different karyotype morphology and different genetic sex-determining systems do not suggest a close relationship between *L. mosorensis* and *L. horvathi*, and this is in contrast to the data of Méhely (1904) and Arnold (1973) based on morphological and osteological features. Incongruence between karyological and anatomical data on one hand, and the fact that *L. mosorensis* and *L. horvathi* have similar ecological strategies (see Bischoff, 1984*a,b*) on the other hand, presumably indicate that the morphological affinities between these species could be interpreted as a by-product of evolutionary convergence. In regard to this must be pointed out, as stated by Arnold (1973), that «many of the external and cranial features used in 'classical' lacertid systematics appear to be potentially labile and therefore must be given low comparative weight in judging relationships».

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References

Arnold E. N., 1973. *Relationships of the palaearctic lizards assigned to the genera* Lacerta, Algyroides *and* Psammodromus *(Reptilia: Lacertidae)*. Bull. Br. Mus. nat. Hist. (Zool.), 25 (8): 291-366.

Arnold E. N., Burton J. A., Ovenden D. W., 1978. *Field Guide to the Reptiles and Amphibians of Britain and Europe*. William Collins Sons, London.

Bhatnagar A. N., Yoniss Y. Th., 1976. *A proof of female heterogamety in a lacertid lizard*, Ophisops elegans. Cytologia, 41: 507-511.

Bischoff W., 1984*a*. Lacerta horvathi *Méhely 1904 - Kroatische Gebirgseidechse*. In: W. Böhme (ed.), *Handbuch der Reptilien und Amphibien Europas*, Band 2/I Echsen (Sauria) II, (Lacertidae II: *Lacerta*). AULA Verlag, Wiesbaden: 265-275.

Bischoff W., 1984*b*. Lacerta mosorensis *Kolombatović 1886 - Mosor Eidechse*. In: W. Böme (ed.), *Handbuch der Reptilien und Amphibien Europas*, Band 2/I Echsen (Sauria) II, (Lacertidae II: *Lacerta*). AULA Verlag, Wiesbaden: 290-300.

CAPULA M., LAPINI L., CAPANNA E., 1989. *The karyotype of* Lacerta horváthi *Méhely, 1904 (Reptilia, Sauria, Lacertidae).* Genetica, 79: 11-16.

CAPULA M., LUISELLI L., 1990. *Notes on the occurrence and distribution of* Lacerta horvathi *Méhely, 1904 in Federal Republic of Germany.* The Herpetological Journal, 1: 535-536.

CAPULA M., NASCETTI G., CAPANNA E., 1982. *Chromosome uniformity in Lacertidae: new data on four Italian species.* Amphibia-Reptilia, 3: 207-212.

DAREVSKY I. S., KUPRIYANOVA L. A., 1982. *Rare males in parthenogenetic lizard* Lacerta armeniaca *Méhely.* Vert. Hung., 21: 69-75.

DE LUCA N., DULIĆ B., 1988. *Karyotype of horvath's rock lizard* Lacerta (Archaeolacerta) horvathi *Méhely, 1904 (Reptilia: Lacertidae).* Amphibia-Reptilia, 9: 353-356.

DE SMET W. H. O., 1981. *Description of orcein stained karyotypes of 36 lizard species (Lacertilia, Reptilia) belonging to the families Teiidae, Scincidae, Lacertidae, Cordylidae and Varanidae (Autarchoglossa).* Acta Zool. Pathol. Antverpiensia, 76: 73-118.

EVANS E. P., BRECKON G., FORD C. E., 1964. *An air-drying method for meiotic preparation from mammalian testes.* Cytogenetics, 3: 289-294.

GORMAN G. C., 1969. *New chromosome data for 12 species of lacertid lizards.* J. Herpet., 3 (1-2): 49-54.

GORMAN G. C., 1973. *The chromosomes of the Reptilia, a cytotaxonomic interpretation.* In: A. B. CHIARELLI, E. CAPANNA (eds.), *Cytotaxonomy and vertebrate evolution.* Academic Press, London - New York: 349-424.

GUILLAUME C.-P., LANZA B., 1982. *Comparaison electrophorétique de quelques espèces de Lacertidés méditerranéens, Genera* Podarcis *et* Archaeolacerta. Amphibia-Reptilia, 4: 361-375.

IVANOV V. G., BOGDANOV O. P., ANISIMOVA E. Y., FEDOROVA T. A., 1973. *Studies of the karyotypes of three lizard species (Sauria, Scincidae, Lacertidae).* Tsitologiya, 15: 1291-1296.

IVANOV V. G., FEDOROVA T. A., 1970. *Sex heteromorphism of chromosomes in* Lacerta strigata *Eichwald.* Tsitologiya, 12: 1582-1585.

IVANOV V. G., FEDOROVA T. A., 1973. *Heterochromosomes in the karyotype of* Eremias arguta *Pall.* Tsitologiya, 15: 762-765.

LANZA B., CEI J. M., CRESPO E. G., 1977. *Immunological investigations on the taxonomic status of some Mediterranean lizards (Reptilia, Lacertidae).* Monit. zool. ital., (N.S.), 11: 211-221.

LAPINI L., DOLCE S., 1983. Lacerta (Archaeolacerta) horvathi *Méhely, 1904 in Italia: nuove stazioni per le Alpi Carniche e Giulie.* Gortania, Atti Mus. Friul. St. Nat., 4 (1982): 213-225.

LUTZ D., BISCHOFF W., MAYER W., 1986. *Chemosystematische Untersuchungen zur Stellung von* Lacerta jayakari *Boulenger, 1887 sowie der Gattungen* Gallotia *Boulenger und* Psammodromus *Fitzinger (Sauria; Lacertidae).* Z. zool. Syst. Evolut.-forsch., 24: 144-157.

LUTZ D., MAYER W., 1985. *Albumin evolution and its phylogenetic and taxonomic implications in several lacertid lizards.* Amphibia-Reptilia, 6: 53-61.

MAYER W., TIEDEMANN F., 1982. *Chemotaxonomical investigations in the collective genus* Lacerta *(Lacertidae: Sauria) by means of protein electrophoresis.* Amphibia-Reptilia, 2: 349-355.

MÉHELY L. VON, 1904. *Eine neue* Lacerta *aus Ungarn.* Ann. hist.-nat. Mus. natn. hung., 2: 362-377.

MÉHELY L. VON, 1910. *Weiter Beiträge zur kenntnis der Archaeo und Neolacerten (als Erwirderung an Herrn G. A. Boulenger).* Ann. hist.- nat. Mus. natn. hung., 8: 217-230.

OLMO E., 1986. *A. Reptilia.* In: B. JOHN (ed.), *Animal Cytogenetics,* vol. 4, *Chordata* 3. Gebrüder Borntraeger, Berlin-Stuttgart.

OLMO E., COBROR O., MORESCALCHI A., ODIERNA G., 1984. *Homomorphic sex-chromosomes in the lacertid lizard* Takydromus sexlineatus. Heredity, 53 (2): 457-459.

OLMO E., ODIERNA G., CAPRIGLIONE T., 1987. *Evolution of sex chromosomes in lacertid lizards.* Chromosoma, 96: 33-38.

OLMO E., ODIERNA G., COBROR O., 1985. *Variazioni cromosomiche inter- e intraspecifiche nei lacertidi.* Atti Soc. Tosc. Sc. Nat., Mem., Serie B, 92: 311-318.

OLMO E., ODIERNA G., COBROR O., 1986. *C-band variability and phylogeny of Lacertidae.* Genetica, 71: 63-74.

SINGH L., PURDOM I. F., JONES K. W., 1976. *Satellite DNA and evolution of sex chromosomes.* Chromosoma, 59: 43-62.

SINGH L., PURDOM I. E., JONES K. W., 1980. *Sex chromosomes associated satellite DNA: evolution and conservation.* Chromosoma, 79: 137-157.

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