Robertsonian Fusions Differentiate Karyologically the Populations of the Pyrenean Rock Lizards (Lacertidae, *Archaeolacerta*)

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Abstract. A karyological study of male and female specimens of different populations of Pyrenean rock lizards was conducted by conventional C-, Ag-NOR, DAPI and Alu I banding methods. These lacertid lizards show karyotypes rich in biarmed elements and peculiar sex chromosomes systems. In fact, *Lacerta aurelioi* possesses a 2n=26 macrochromosomes (10 biarmed + 12 acrocentric), the NOR localised on the long-armed telomeres of the third pair of homologues, sex system of type Z_1Z_2W. Whereas, *Lacerta bonnali* (specimens of populations of Macizo de Bigorre - Lac Bleu and Perdido), instead, possesses a 2n=24 macrochromosomes (12 biarmed + 12 acrocentric), the NOR localised on the long-armed telomeres of the third pair of homologues, a sex system of type Z_1Z_2W. The latter species differs from *Lacerta aurelioi* in the length of the W-chromosomes, as well as in some characteristics of heterochromatin. Furthermore, both males and females belonging to populations ascribed to *Lacerta bonnali aranica* possess a 2n=26 macrochromosomes (10 biarmed + 12 acrocentric), the NOR localised on the long-armed telomeres of the third pair of homologues. Lastly, the specimens of populations of the Macizo de Posets belonging to *Lacerta bonnali bonnali* possess a 2n=22 all biarmed chromosomes. Differences and similarities among these Pyrenean rock lizards are discussed.

Introduction

In the Pyrenean area two distinct species have been identified, *Lacerta aurelioi* Arribas, 1994, widespread in the Eastern Pyrenees from the Macizo de Montroig to Andorra, and *Lacerta bonnali* Lantz, 1927 (Arribas, 1993a, Perez-Mellado *et al.*, 1993). The different populations of the latter species display a relevant intraspecific variability and two distinct subspecies have been identified: *Lacerta bonnali bonnali* which is located in the Central and Western Pyrenees and *Lacerta bonnali aranica* Arribas, 1993, with a range from north Aràn mountains to Mont Vallier Massif (Arribas, 1993 a,b).

Karyological studies have significantly contributed to the knowledge of phylogeny and systematics of lacertids. Therefore we undertook a karyological study of specimens from different populations of Pyrenean rock lizards.

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Materials and Methods

The number, sex and origin of specimens of two Pyrenean species are indicated in table 1. Chromosome preparations were obtained from bone marrow, intestine, gonads and spleen using the techniques described by Olmo *et al.* (1986) and were stained by a 5% Giemsa solution in phosphate buffer pH 7.O. C-bands were produced with the technique of Sumner (1972) using a $Ba(OH)_2$ treatment at 40° C. AgNOR staining followed Howell and Black (1980), and staining with DAPI was carried out according to the method of Schweizer (1976). Digestion with Alu I restriction enzyme followed Mezzanotte *et al.* (1983). The preparations were digested for 16 hr in a solution of the enzyme at a concentration of 30 units/100 µl of the buffer recommended by the supplier.

Table 1. Summary of the matherials used. All the specimens were collected by Arribas. The specimens that were not destroyed during the DNA extraction were deposited in the Hepetological Collection of Dr. V. Caputo, Ancona.

Species	populations	sex	N	Voucher
L. aurelioi	Massif de Montroig	male	1	1116
	Estany de Barbote, Pica d'Estats	female	3	1105
	Estany de Sotllo, Pica d'Estats	male	1	1109
		female	1	1110
	Andorra	male	1	1112
		female	2	1113 - 14
L. bonnali bonnali	Monte Perdido	male	2	1144 - 45
		female	3	1146 - 48
	Bigorre - Lac blue	male	1	-----
		female	2	-----
	Posets	male	2	-----
		female	1	-----
L. bonnali aranica	Colle de Barradòs, Valle de Aràn	male	1	1118
		female	2	-----
	Tuc de Crabes, Valle de Aràn	male	2	-----
		female	2	-----

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Results

Like *Lacerta monticola*, *Lacerta horvathi* and *Lacerta vivipara* [2n=36 all acrocentric macrochromosomes, number fundamental, *i.e.* the total number of arms, FN=72 (Chevalier *et al.*, 1979, Capula *et al.*, 1989, Odierna *et al.*, 1993, 1995b)] all the specimens of the Pyrenean rock lizards exhibit karyotypes devoid of the microchromosome pair, but, unlike the former, the karyotypes of latter are rich in biarmed elements. Karyotypes rich in biarmed chromosomes, so far, have been found only in *Lacerta parva* and *Lacerta fraasii* (Gorman, 1969, Kupriyanova, 1980, Odierna *et al.* 1995a).

Chromosome morphology

Lacerta aurelioi

The male specimens of the various *Lacerta aurelioi* populations show 26 macrochromosomes (10 biarmed and 16 acrocentric). The 13 bivalents during meiotic diakinesis are here reported in figure 1a). The FN is 72; therefore the karyotype of *Lacerta aurelioi* specimens may be derived from a *Lacerta monticola*-like karyotype by fusion of five pairs of uniarmed elements. The female specimens of this species possess 25 macrochromosomes, 11 of which are biarmed and 14 uniarmed (Fig. 1b). This is clearly due to the presence of sex chromosome system of the Z_1Z_2W type, where W is derived by the fusion of the chromosome-W with an autosome, it is submetacentric and shows an intermediate size between the 4th and 5th pair of homologues. Moreover, the chromosome Z_1 is easily detectable, being the largest among the uniarmed ones. So far, only in the viviparous lacertid, *Lacerta vivipara*, a similar sex chromosome system has been described (Kupriyanova 1990; Odierna *et al.*, 1993; Capriglione *et al.*, 1994a).

Lacerta bonnali

The specimens of the different *Lacerta bonnali* populations show two unique characteristics among lacertids. One characteristic concerns an intraspecific variation due to Robertsonian mutations, which, among saurians, have been found in *Anolis* (Webster *et al.*, 1972), *Cordylus* (Olmo and Odierna, 1980) and *Scelo-*

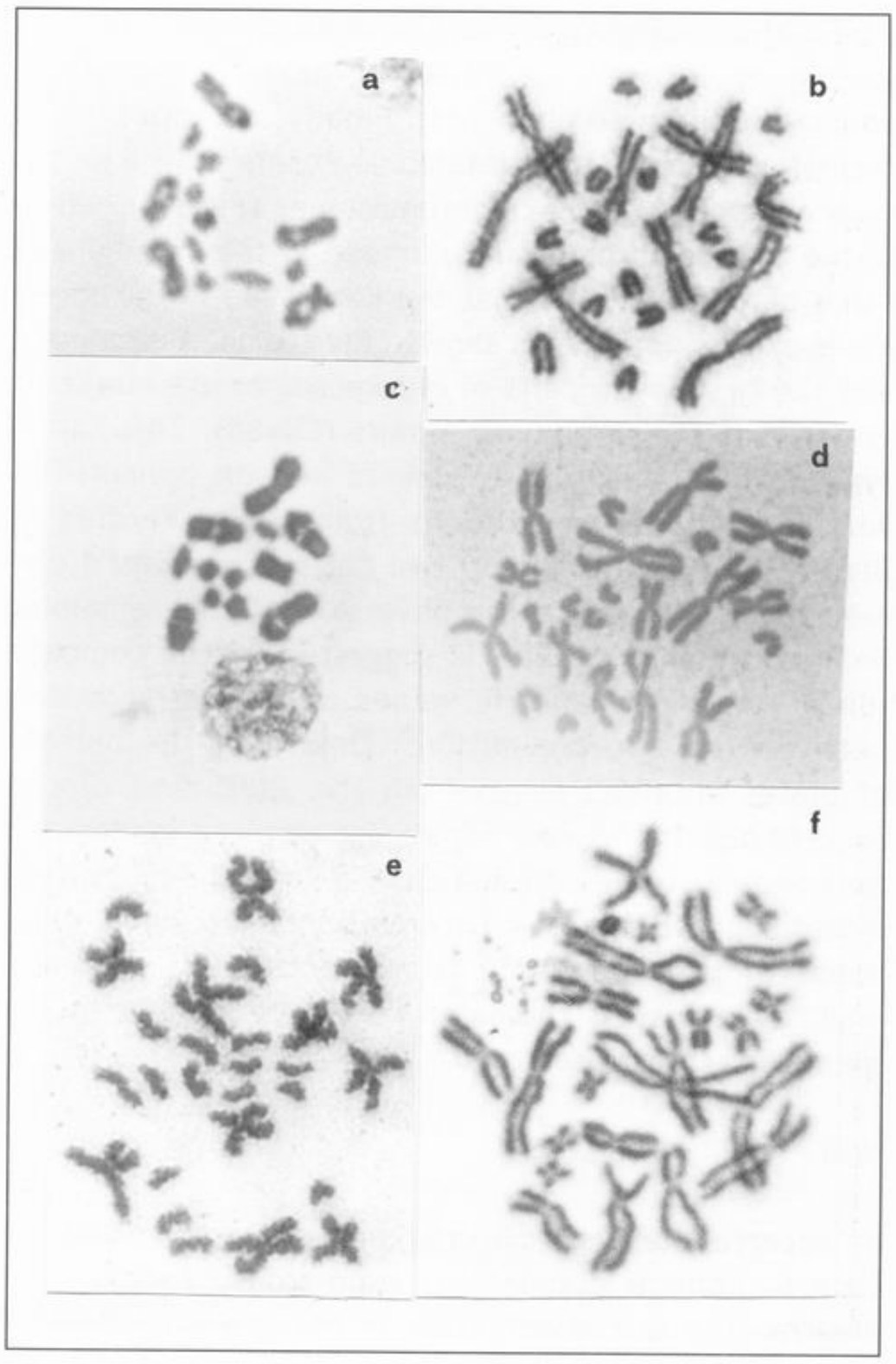

Fig.1 *Male meiosis (a, c) and female metaphase plates (b, d, e, f) of Lacerta aurelioi (a, b), Lacerta bonnali bonnali from monte Perdido and Bigorre (c, d), Lacerta bonnali aranica (e) and Lacerta bonnali bonnali from Posets (f).*

porus (Sites, 1983). In fact, both male and female specimens of the two *Lacerta bonnali aranica* populations show a diploid number 2n=26 macrochromosomes, 10 of which biarmed and 16 acrocentric (FN=72) (Fig.1c).

Male specimens of the *Lacerta bonnali bonnali* population from Monte Perdido and Bigorre (Lac Blue), instead, show a diploid number 2n=24 macrochromosomes, 12 of which biarmed and 12 acrocentric (the 12 bivalents of meiotic plates are here represented) (FN=72) (Fig. 1d). Therefore it is plausible that this karyotype might derive from a *Lacerta bonnali aranica*-like karyotype by fusion between two pairs of uniarmed elements. It is to be pointed out that the female specimens of these *Lacerta bonnali bonnali* populations show a karyotype of 23 chromosomes (Fig 1e), and hence show a sex chromosome system of the Z_1Z_2W type, like *Lacerta aurelioi*. However, in the former the chromosome-W is metacentric and markedly smaller than in the latter

(the chromosome-W of the *Lacerta bonnali bonnali* specimens is easily identifiable being the smallest among the biarmed elements). Finally, *Lacerta bonnali bonnali* specimens from Macizo de Posets show a karyotype composed of 2n=22 chromosomes (Fig. 1f), which is the lowest chromosome number in the family. The other characteristic is that the karyotype in the specimens of this population shows only biarmed elements, and the first seven pairs of chromosomes are markedly larger than the remaining 4 pairs (FN=88). This karyotype might derive from a *Lacerta bonnali bonnali*-like karyotype of the populations from Monte Perdido or Bigorre by fusion between two pairs of uniarmed elements and the pericentric inversion of the remaining uniarmed elements. This is suggested by the comparison of the morphometric values of the chromosomes between the two populations. Unluckily, the number of plates obtained by us from the specimens of this *Lacerta bonnali bonnali* population resulted inadequate for banding studies. In addition, a preliminary analysis would suggest that the *Lacerta bonnali bonnali* specimens of the population from Macizo dela Maladeta might also possess the same karyotype as that of the specimens from Posets.

NOR

In *Lacerta aurelioi*, *Lacerta bonnali aranica* and in *Lacerta bonnali* populations from Monte Perdido and Bigorre (the last observation is not shown), the NOR is telomerically located on the long arm of the 3rd pair of homologues (Fig. 2) This arm has similar sizes to that of a NOR-bearing chromosome (L-type according to Olmo *et al.*, 1993) found in several species of *Lacerta* [*Lacerta bediragae*, *Lacerta horvathi*, *Lacerta monticola*, *Lacerta graeca*, *Lacerta caucasica*, *Lacerta dahli*, *Lacerta mixta*, *Lacerta saxicola*, *Lacerta viridis*, *Lacerta trilineata*, *Lacerta agilis*, *Lacerta schreiberi* (Olmo *et al.*, 1993, Odierna *et al.*, 1995b)].

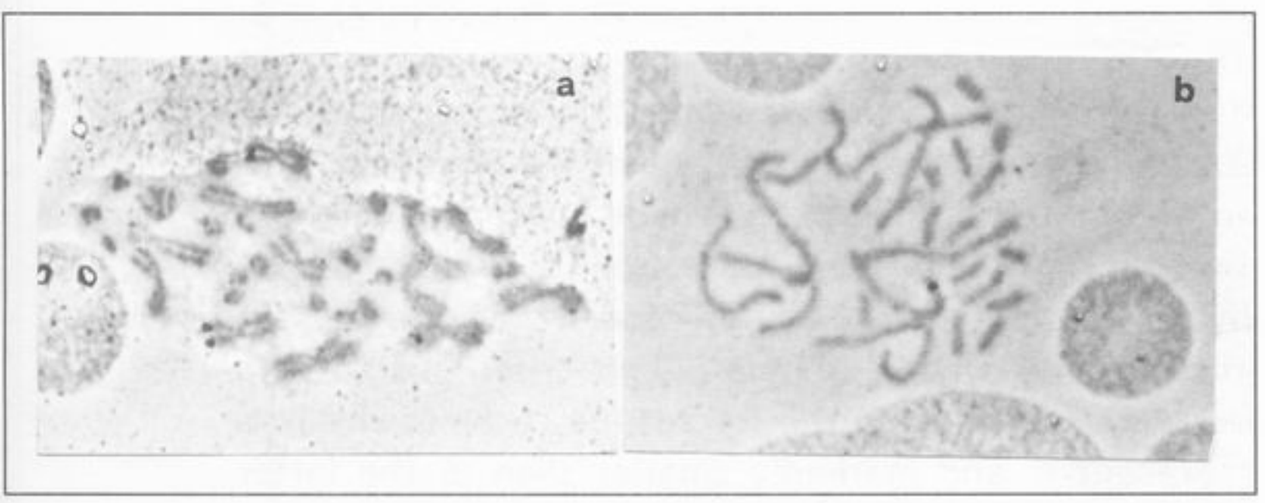

Fig.2
Ag-NOR stained metaphase plates of Lacerta aurelioi (a) and Lacerta bonnali aranica (b).

Heterochromatin

C-banding reveals that in *Lacerta aurelioi* uniarmed chromosomes show intense centromeric bands, and biarmed chromosomes show thin centromeric bands (Fig. 3a). Faint pericentromeric bands are present among the first three pairs of biarmed chromosomes. Interestingly, the centromeric bands both in uniarmed and biarmed chromosomes are digested with Alu I (Fig 3b), whereas pericentromeric bands are resistant to it. Neither band selectively stains with DAPI (Fig. 3c)

In the *Lacerta bonnali* specimens of the population from Monte Perdido, most heterochromatic material is sex-associated. In fact, only the chromosome-W shows an intense heterochromatic band subtelomerically located on either arm (Fig. 3d). This sex chromatin is DAPI-negative (Fig. 3e) and Alu I-sensitive (Fig. 3f).

The biarmed chromosomes of the *Lacerta bonnali aranica* specimens also show thin centromeric and pericentromeric bands of heterochromatin, whereas telocentric chromosomes show intense centromeric bands (Fig 3g). The pericentromeric bands of the first 3 metacentric, the NOR-associated band and the pericentromeric band of the 7th pair are resistant to the digestion with Alu I (Fig. 3 h); the last is DAPI-positive (Fig. 3 i).

Fig. 4 summarises similarities and differences among the karyotypes of the various Pyrenean populations.

Discussion

The populations of the various taxa are different in at least one Robertsonian mutation involving fusion between the autosomes and/or one autosome with the heterochromosome W. The data on heterochromatin composition and distribution also appear quite interesting. Heterochromatin is a genomic material with well-defined evolutionary dynamics (Charlesworth *et al.*, 1994). Therefore, the pattern of the variations in the repetitive sequences making up this genomic material can provide information on the phylogeny and systematics of many organisms, including lacertids (Capriglione *et al.*1991, 1994b). In *Lacerta aurelioi*, *Lacerta bonnali aranica*, and *Lacerta bonnali bonnali* populations from Monte Perdido and Bigorre, the great differentiation in both localisation and composition of this genomic

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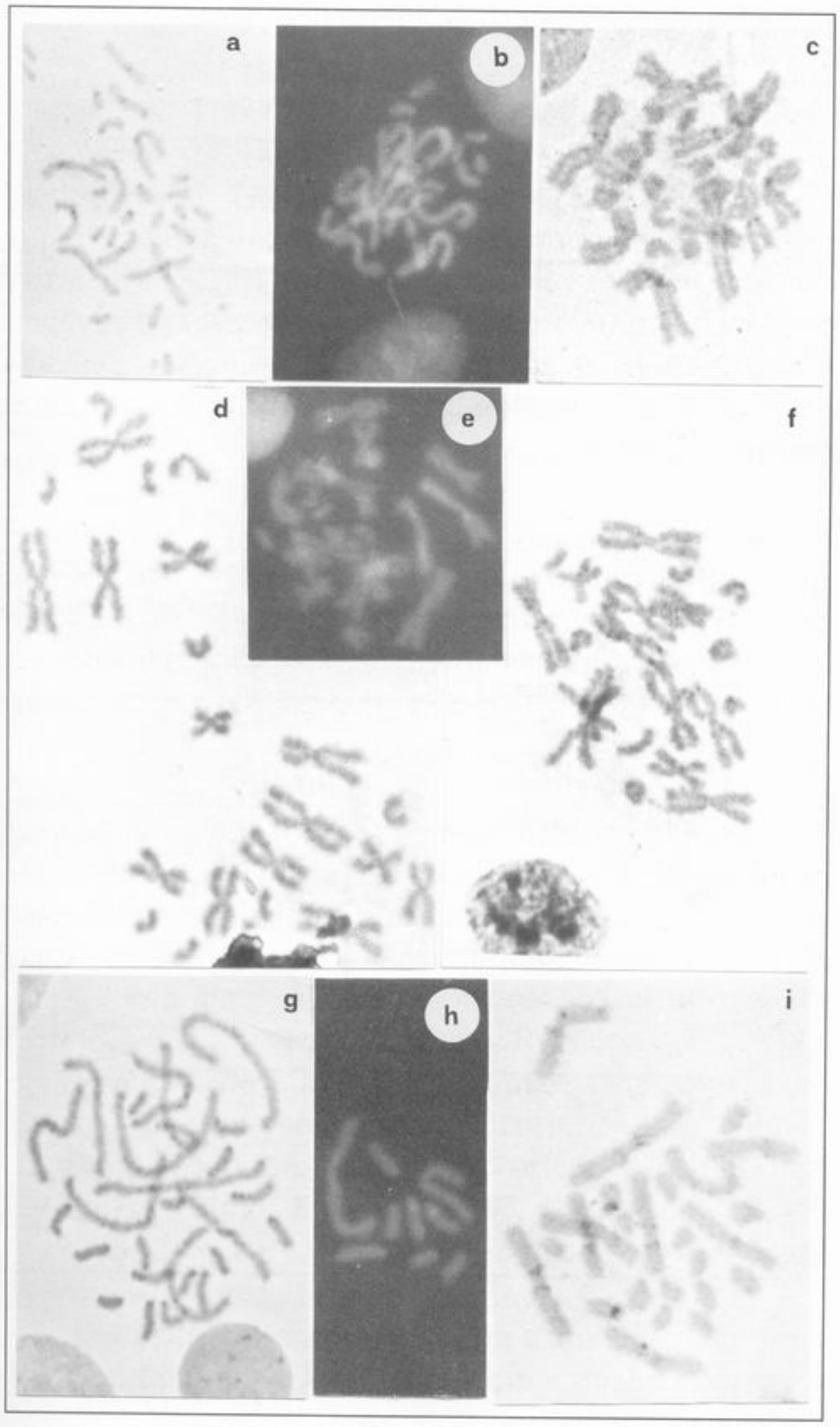

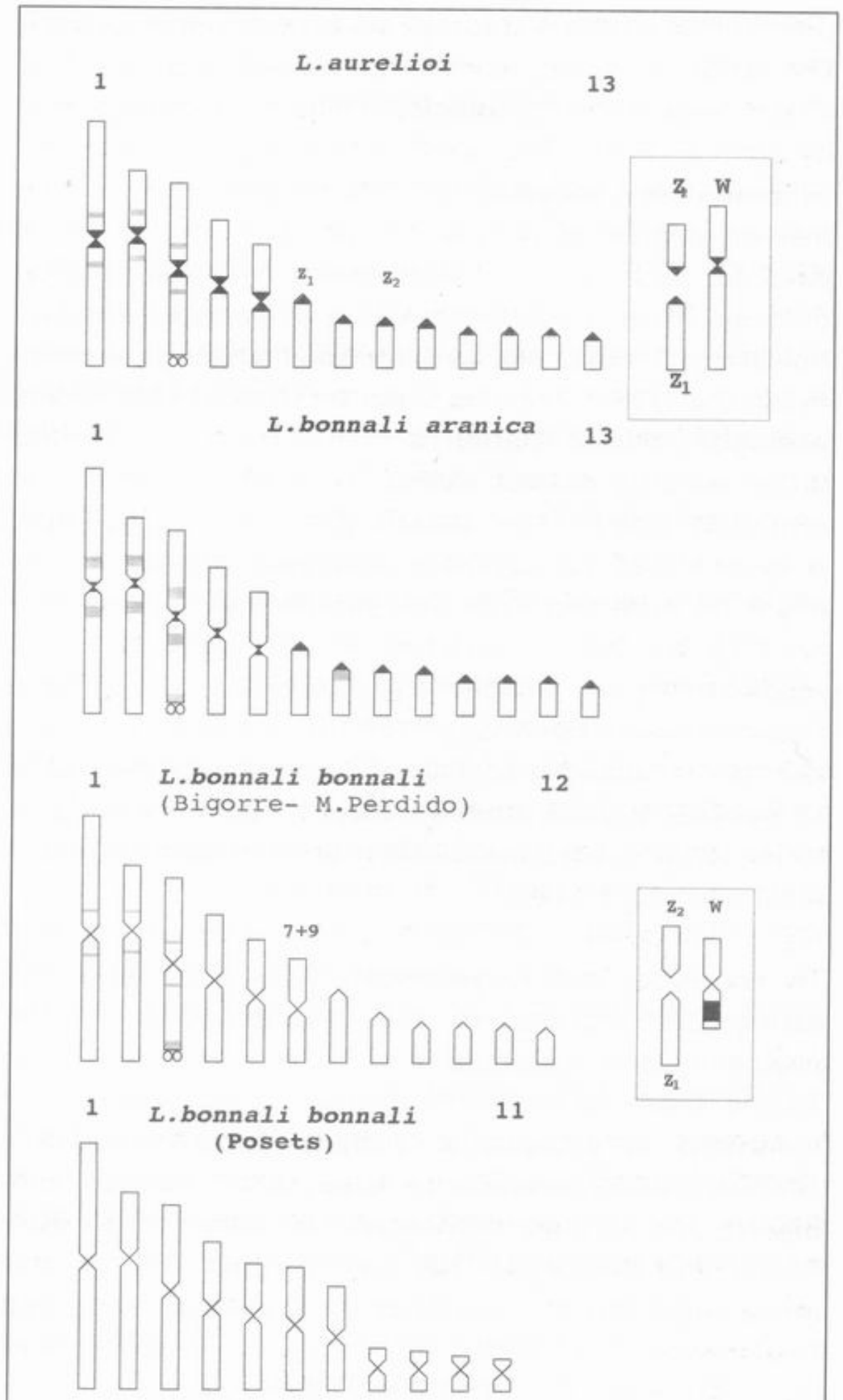

Fig.4 *Haploid idiogram of Lacerta aurelioi and populations of Lacerta bonnali. Solid black boxes, circles, grey solid boxes show the C-, AgNOR and Alu I banding pattern, respectively.*

material would suggest a remote isolation of these species, which may have favoured the Robertsonian mutations observed and their fixation. In this regard, the data concerning heterochromatin distribution in *Lacerta bonnali aranica* specimens and in the specimens of the nominal subspecies of the population from Bigorre and Monte Perdido would suggest the involvement of this genomic material in inducing or favouring the Robertsonian mutation. A peculiar type of DAPI-positive AluI-resistant heterochromatin is exclusively and significantly present proximally to the centromeres of the 7th pair of acrocentric chromosomes in *Lacerta bonnali aranica*. (It is noteworthy that, on the basis of morphometric analysis, the additional pair of biarmed chromosomes of the karyotype found in *Lacerta bonnali bonnali* specimens from Bigorre and Monte Perdido would derive by the fusion of acrocentrics having the same size as those of the 7th and 9th pair of homologues present in the karyotype of *Lacerta bonnali aranica* and *Lacerta aurelioi*). On this basis, it can be hypothesised that the Alu I-resistant DAPI-positive pericentromeric heterochromatin might have originated in the 7th pair of homologues in *Lacerta bonnali aranica* populations after isolation from the populations of the nominal subspecies, thus preventing the centric fusion between this pair of homologues and the 9th pair. This fusion, instead, would have occurred in the specimens of the *Lacerta bonnali bonnali* population from Monte Perdido and Bigorre.

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Interestingly, the karyological differentiation among the various populations of Pyrenean rock lizards is consistent with the morphological differentiation reported by Arribas (1993b; Fig. 5). It is to be pointed out that, in some cases, karyological data suggest a much more marked degree of differentiation than morphological data do, such as in the populations from Bigorre-Perdido and Posets-La Maladeta of *Lacerta bonnali bonnali*. However, this may not be surprising, since, as stressed by Arnold (1989: 244) "in some sections of Lacertidae, ecological differentiation is rather subtle ... Species differ in fairly modest aspects of spatial niche and in relatively small-scale climatic parameters, and there is evidence of considerable ecological parallelism. As might be expected from this, morphological variations tend to be fairly restricted and homoplasious, and reconstruction of phylogeny is often difficult". In addition, it has also been suggested that, in some Antarctic fish species showing ecological and morphological similarity, but very different karyotypes, karyological evolution has preceded many other forms of differentiation (Eastman, 1993:119).

To conclude, our karyological study provides interesting contributions to the understanding of the phylogeny and systematics of Pyrenean rock lizards. Among them four clearly distinct groups have been identified karyologically, namely *Lacerta aurelioi*, *Lacerta bonnali aranica*, *Lacerta bonnali bonnali* from Bigorre and Perdido, and *Lacerta bonnali bonnali* from Posets and Maladeta. This suggests that this lacertid group might include more than the presently recognised two species.

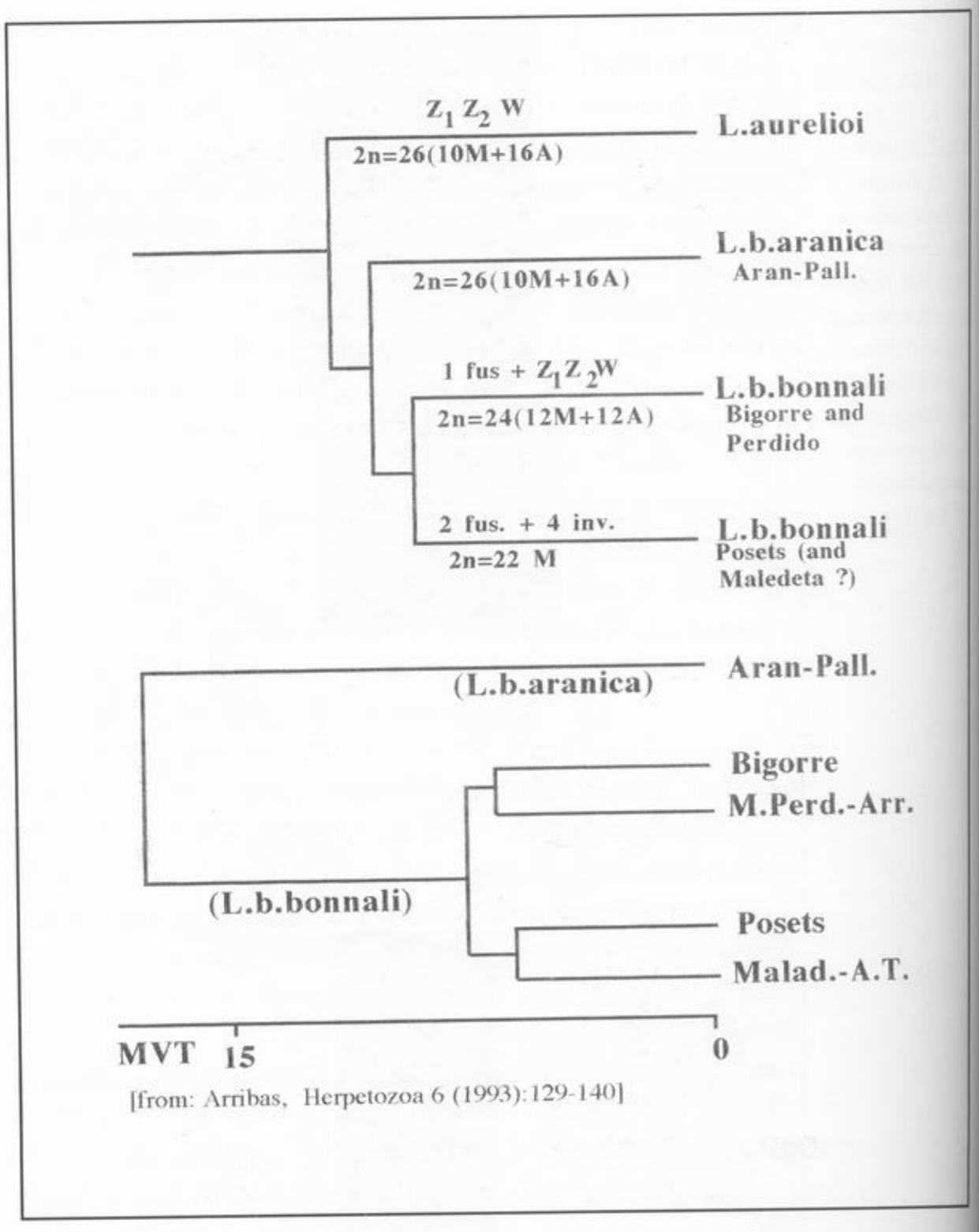

Fig.5 Comparison among karyological differentiation (above) and morphological differentiation of the Pyrenean rock lizards. Solid black boxes, circles, grey solid boxes show the C-, AgNOR and Alu I banding pattern, respectively.

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