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THE KARYOLOGY OF THE IBERIAN ROCK LIZARDS

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ABSTRACT: We conducted a karyological study of Iberian rock lizards by standard and C-, AgNOR-, DAPI-, and Alu I-banding methods. The results showed that *Lacerta monticola monticola* and *L. m. cantabrica* possess 2N = 36 uniarmed chromosomes. *Lacerta m. cyreni* differs from the other two subspecies of *L. monticola* in the NOR-bearing chromosome pairs and in the presence of the heteromorphic and heterochromatic W-chromosome. The Pyrenean species *L. aurelioi* and *L. bonnali* differ from *L. monticola* in having a karyotype rich in biarmed elements. In fact, *L. aurelioi* possesses a 2N = 26 karyotype with 10 biarmed + 16 uniarmed macrochromosomes. In *L. bonnali*, an intraspecific variability in diploid number due to Robertsonian translocations was found: the specimens of *L. b. aranica* have a karyotype similar to that of *L. aurelioi* while the specimens from nominal subspecies (population of Monte Perdido) possess a 2N = 24 karyotype with 12 biarmed + 12 uniarmed macrochromosomes. Our results confirm that Iberian rock lizards form a heterogeneous group in which at least five taxa can be distinguished karyologically.

Key words: Karyology; C-banding; Chromosome; Robertsonian polymorphism; Iberian *Archaeolacerta*; Lacertidae

TRADITIONALLY the Iberian rock lizards have been assigned to the single species *Lacerta* (*Archaeolacerta*) *monticola* (Boulenger, 1905). However, recent morphological studies have demonstrated that these lizards, particularly the Pyrenean populations, form a heterogeneous group, and some populations are distinct at the specific level (Arribas, 1993*a*; Perez-Mellado et al., 1993). In the Pyrenean area, two distinct species have been identified: *L. aurelioi* and *L. bonnali*; they differ in several morphological characters, including osteology of the skull and of the pectoral girdle and hemipenial microornamentation (Arribas, 1993*a*, 1994). Arribas (1993*b*) has also observed considerable intraspecific variability in the morphology of various populations of *L. bonnali* and identified two distinct subspecies: *L. b. aranica* with a range bordering that of *L. aurelioi* and *L. b. bonnali* which is distributed in the central and western Pyrenees.

Karyological studies have significantly contributed to the knowledge of phylogeny and systematics of lacertids (Olmo et al., 1990, 1993). Therefore we undertook a karyological study of specimens from different populations of Iberian rock lizards ascribed to different subspecies of *L. monticola* (*L. m. cantabrica*, *L. m. cyreni*, and *L. m. monticola*) and to the two species from the Pyrenees, using conventional and banding techniques. The purpose of this research was to verify whether karyological studies can provide additional information regarding species boundaries among the various populations of Iberian rock lizards.

MATERIALS AND METHODS

Number, sex, and source of the specimens examined are listed in Appendix I. We obtained chromosome preparations from bone marrow, intestine, gonads, and spleen using the techniques described by Olmo et al. (1986), and we stained them by a 5% Giemsa solution in phosphate buffer. 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). Di-

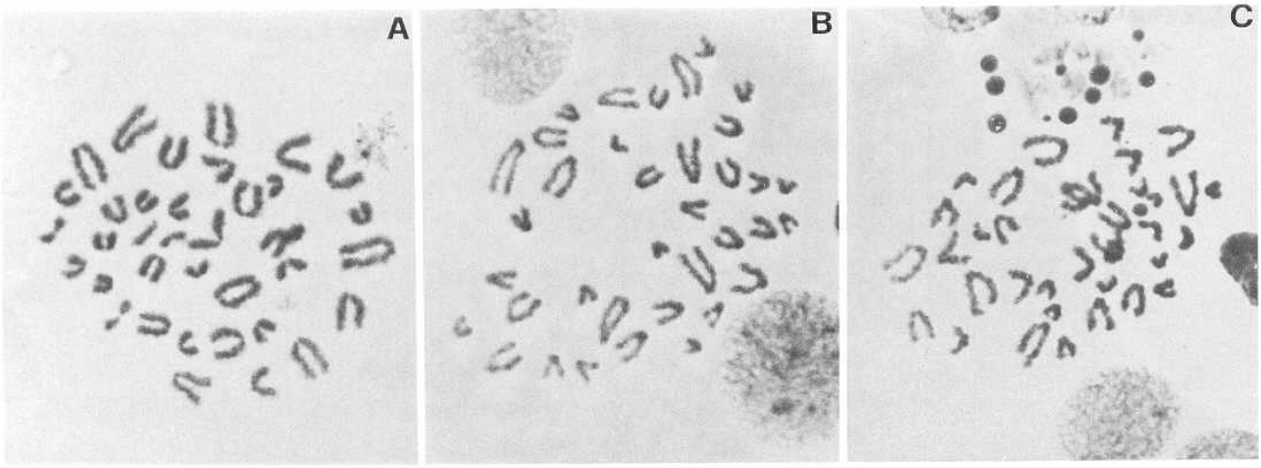

FIG. 1.—Metaphase plates of (A) *L. m. cantabrica*, (B) *L. m. monticola* (1101 HCC), and (C) *L. m. cyreni* (1100 HCC).

gestion with the restriction enzyme Alu I followed Mezzanotte et al. (1983). The preparations were digested for 16 h in a solution of the enzyme at a concentration of 30 units/100 μl of the buffer recommended by the supplier.

RESULTS

All of the Iberian rock lizards showed a karyotype devoid of microchromosomes. The specimens assigned to the subspecies *Lacerta m. cantabrica* and *L. m. monticola* displayed the same karyotype with 36 gradually decreasing uniarmed macrochromosomes (Fig. 1A,B) showing clear centromeric C-bands (Fig. 2A,C). Digestion with Alu I removed the centromeric C-bands and produced pericentromeric bands on the first six pairs of homologs (Fig. 2B,D). The NOR was localized on the telomere of a pair of large chromosomes corresponding to the L-type NOR-bearing ones (after Olmo et al., 1990) found in several species of *Archaeolacerta* and *Lacerta* sensu stricto according to Arnold (1973) (Odierna et al., 1987; Olmo et al., 1993) (Fig. 3A,B).

The specimens ascribed to *L. m. cyreni* also possessed 36 uniarmed macrochromosomes (Fig. 1C), but they differed in NOR localization, C-banded sex chromosomes, and, to some extent, heterochromatin content. In this subspecies, NOR was localized subtelomerically on a pair of medium–small sized chromosomes corresponding to MS-type NOR-bearing ones (after Olmo et al., 1990) found in several lacertid species (Fig. 3C). C-banding revealed centromeric bands (Fig. 2E) that were digested by Alu I (Fig. 2F) and stained with DAPI (Fig. 2G). In addition C-banding showed female heterogamety of the ZW type, where W was smaller than Z and completely heterochromatic except an intercalary euchromatic band (Fig. 2E). W heterochromatin was digested by the Alu I enzyme (Fig. 2F) and was positive to DAPI (Fig. 2G). In this lizard, treatment with Alu I yielded pericentromeric bands identical to those observed in *L. m. cantabrica* and *L. m. monticola* (Fig. 2F).

Both NOR localization and the heterochromatic W-chromosome of *L. m. cyreni* are consistent with those already described in specimens from a different population (Sierra de Gredos) of the same subspecies (Olmo et al., 1987).

Mitotic plates of male specimens of *L. aurelioi* displayed 26 macrochromosomes, 10 of which were biarmed and 16 uniarmed; meiotic plates showed 13 bivalents (Fig. 4D). Female specimens possessed 25 macrochromosomes, 11 biarmed and 14 uniarmed (Fig. 4A). This is clearly due to the occurrence of sex chromosomes of the Z_1Z_2W type, where W was submetacentric and Z_1 was easily recognizable, being the largest acrocentric chromosome (Fig. 4A).

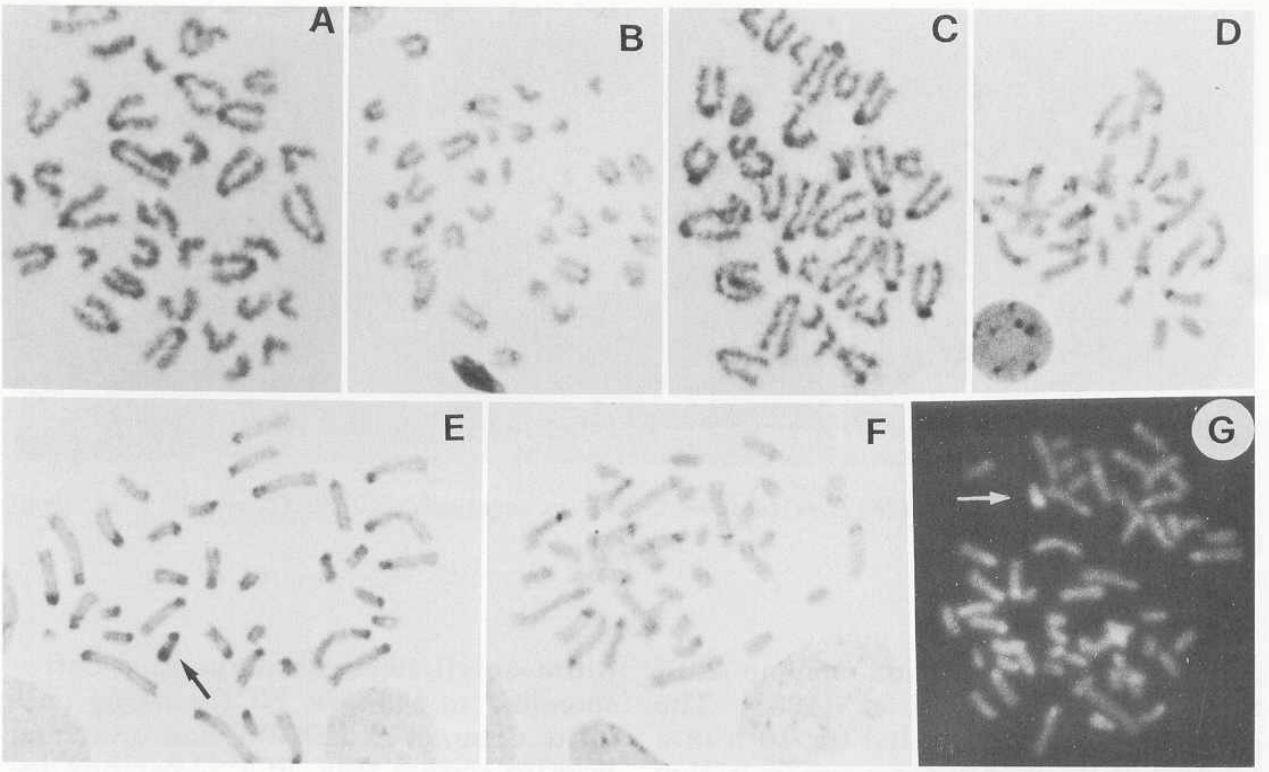

FIG. 2.—(A, C, E) Female C-banded metaphase plates, (B, D, F) Alu-I digested, and (G) DAPI stained of (A, B) *L. m. monticola*, (C, D; 1105 HCC) *L. m. cantabrica*, and (E, F, G) *L. m. cyreni*: arrows indicate W chromosome; 1100 HCC.

C-banding showed centromeric bands that were intense on acrocentric chromosomes and very faint on the biarmed ones. Faint pericentromeric C-bands were present on the first three pairs of biarmed chromosomes (Fig. 5A). The centromeric bands were digested by Alu I, whereas the pericentromeric ones were resistant to it (Fig. 5C). Neither type of band was selectively stained by DAPI (Fig. 5B). The NOR was localized telomerically on the long arm of the 3rd biarmed chromosome (Fig. 3D) which had a size similar to that of the NOR-bearing chromosome of *L. m. cantabrica* and *L. m. monticola*.

Arribas (1993*b*) ascribed the population of *L. bonnali* from Monte Perdido to the subspecies *L. b. bonnali*. The mitotic plates of the male specimens showed 24 macrochromosomes, 12 biarmed and 12 uniarmed, and meiotic plates showed 12 bivalents (Fig. 4E). As with *L. aurelioi*, the female specimens of this population had sex chromosomes of the Z_1Z_2W type. In fact, in mitotic plates of females, 23 chromosomes were counted: 13 biarmed and 10 uniarmed (Fig. 4B). The W chromosome was easily recognizable being the smallest biarmed chromosome, and differed from that of *L. aurelioi* in being smaller and metacentric (Fig. 4B) and in having an evident C-band on one arm at a subtelomerical position (Fig. 5D). Faint pericentromeric bands were present in the first three biarmed chromosomes that appeared resistant to Alu I (Fig. 5F). This enzyme, instead, revealed an intense telomeric band on a pair of biarmed chromosomes, probably corresponding to the NOR-bearing ones (Fig. 5F). In fact, Ag stained the telomeres of the long arm of the 3rd pair of homologs (data not shown). DAPI staining was uniform on all the chromosomes (Fig. 5E).

Unlike the nominal subspecies, both male and female specimens from the population of Coll de Barrados, ascribed to the subspecies *L. b. aranica* by Arribas (1993*b*), showed metaphase plates with 10 biarmed and 16 acrocentric macrochromosomes,

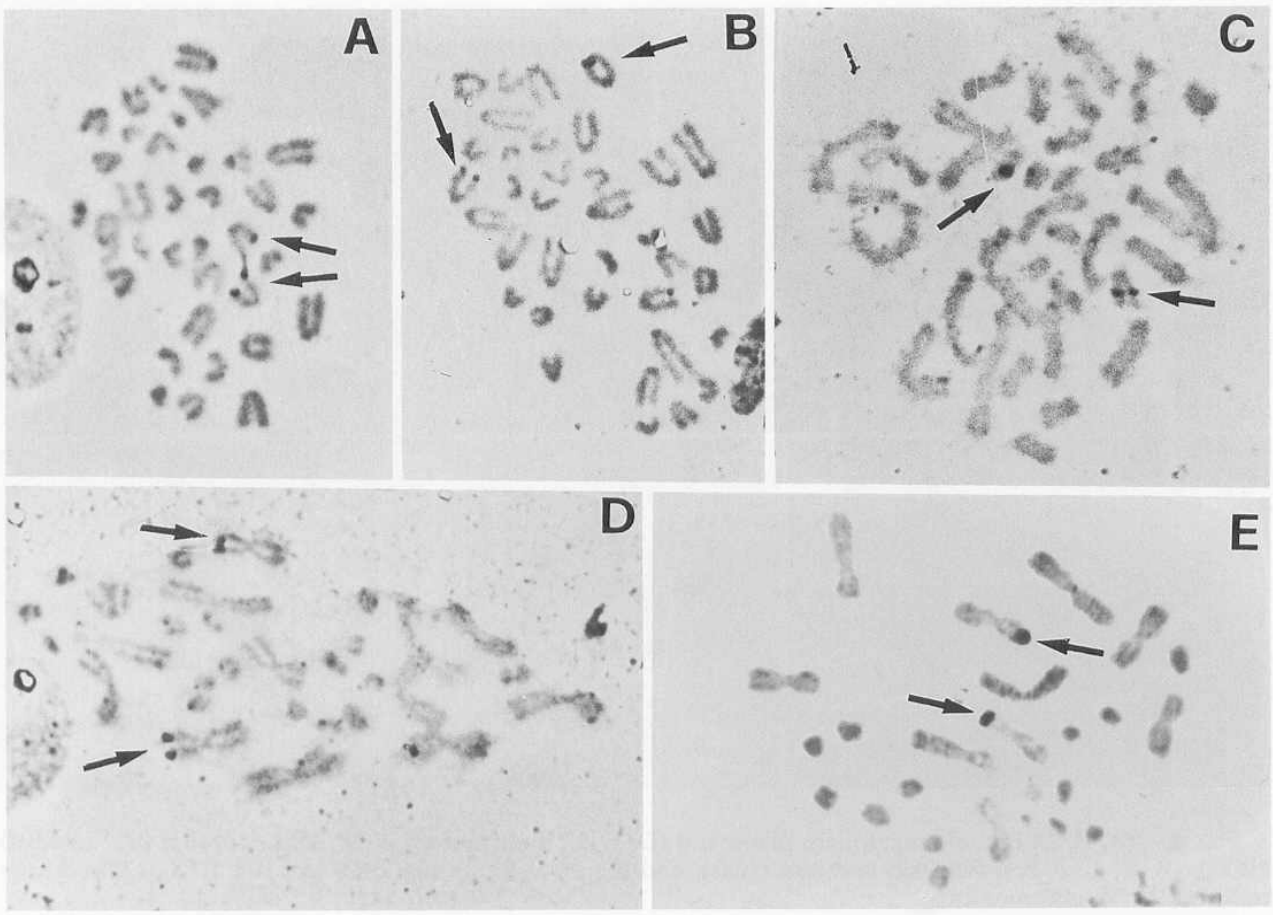

FIG. 3.—Ag-NOR stained metaphase plates of (A) *L. m. cantabrica* (1105 HCC), (B) *L. m. monticola*, (C) *L. m. cyreni* (1100 HCC), (D) *L. aurelioi* (910 HCC), and (E) *L. b. aranica* (971). The arrows identify NOR-bearing chromosomes.

similar to that of *L. aurelioi* in standard morphology (Fig. 4C), and meiocytes with 13 bivalents (Fig. 4F). A convenient number of plates to perform banding stainings were obtained only for the male specimens of this population. Hence it was not possible to detect whether differentiated sex chromosomes are present in this subspecies.

The NOR was localized at the telomeres of the long arm of the 3rd pair of homologs (Fig. 3E), as in the nominal subspecies and *L. aurelioi*. C-banding revealed intense centromeric bands only on uniarmed chromosomes, and faint centromeric and pericentromeric bands on the biarmed ones (Fig. 5G). Centromeric bands were completely digested with Alu I, while the pericentromeric bands of the first three biarmed chromosomes, the NOR-associated subtelomeric band, and an intense pericentromeric band of the 7th pair of (acrocentric) chromosomes were resistant to it (Fig. 5I). Only the last band was positive to DAPI (Fig. 5H).

In addition, a morphometric analysis of the karyotypes of the three taxa from the Pyrenees showed that the corresponding pairs of homologs have the same morphology and relative length, and that the additional pair of metacentrics present in the karyotype of *L. b. bonnali* may be derived by centric fusion of the 7th and 9th acrocentric chromosomes (Fig. 6B). Figure 6A,B summarizes similarities and differences among the karyotypes of the various taxa of Iberian rock lizards.

DISCUSSION

Karyological characters confirm that Iberian rock lizards form a heterogeneous taxon, in which the Pyrenean populations

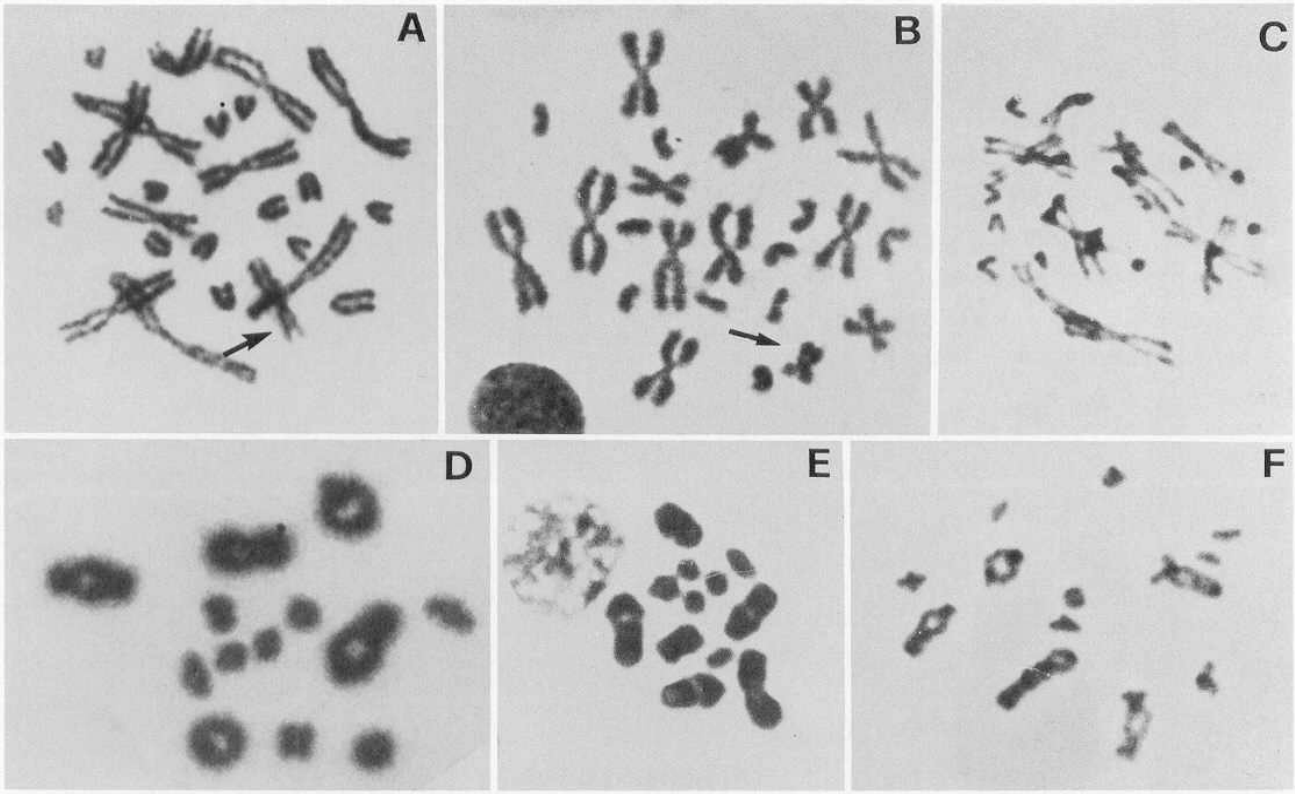

FIG. 4.—(A, B, C) Female metaphase plates and (D, E, F) male meiosis of (A, D) *L. aurelioi* (957 and 910 HCC), (B, E) *L. b. bonnali* (966 and 964 HCC), and (C, F) *L. b. aranica* (969 and 971 HCC). The arrows indicate the W chromosome.

are clearly distinct from *L. monticola*. Moreover, in *L. monticola*, two distinct groups can be recognized on the basis of chromosome morphology. One group includes *L. m. cantabrica* and *L. m. monticola*, which have identical karyotypes. The second group includes *L. m. cyreni* that, though possessing the same diploid number as the other two subspecies of *L. monticola*, is different in NOR localization and the occurrence of a heteromorphic and heterochromatic W chromosome. Both of these characters appear fixed throughout the range of *L. m. cyreni*. Although this taxon has not been considered so far as a distinct species, it is morphologically the most differentiated among the subspecies of *L. monticola* with differences in the color pattern of both adults and hatchlings (Arribas, 1993*a*; Perez-Mellado et al., 1993).

Karyological results are in agreement with these observations and confirm the different taxonomic position of *L. m. cyreni* with respect to the other subspecies. In fact, among the various chromosome characters, NOR localization has proved to have a good diagnostic value in lacertids (Odierna et al., 1987), and cases of intraspecific variation in this character are not known in this family (Olmo et al., 1993). In addition, several authors (Amemiya and Gold, 1988; Foresti et al., 1993; Phillips et al., 1988) have shown that in taxa with generally uniform NOR localization, variation in this character often accompanies events of species differentiation. Thus the karyological differences between *L. m. cyreni* and the other two subspecies support the interpretation that *cyreni* may well be taxonomically distinct at the specific level.

More marked differences are present in the Pyrenean populations, which possess karyotypes rich in biarmed chromosomes and sex chromosomes of the Z_1Z_2W type, where W is biarmed. These characters are quite rare in lacertids. In fact, among the over 90 species of the family that have

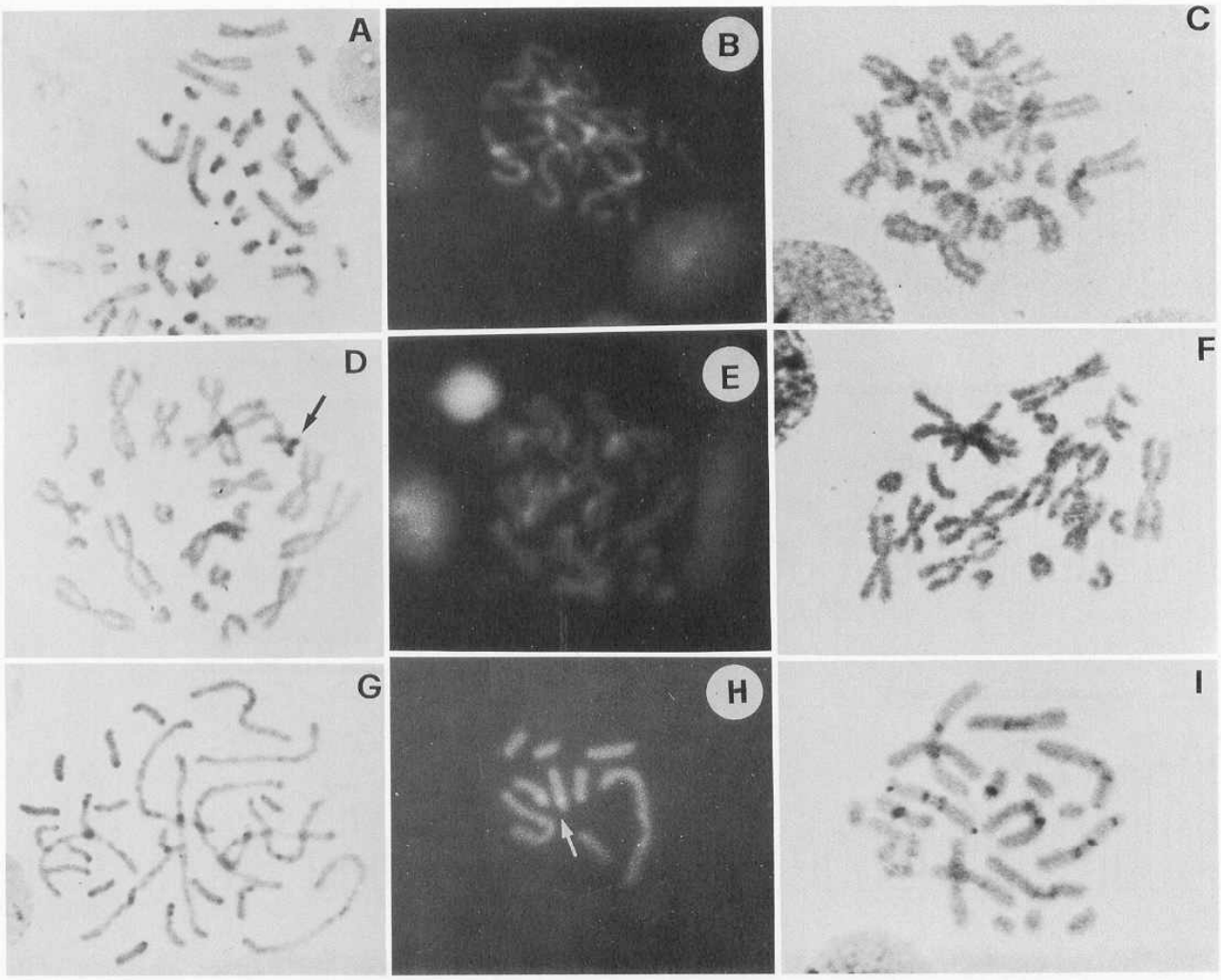

FIG. 5.—(A, D, G) C-banded, (B, E, H) DAPI stained, and (C, F, I) Alu-I digested metaphase plates of (A, B, C) *L. aurelioi* (971 HCC), (D, E, F) *L. b. bonnali* (black arrow indicates W chromosome; 966 HCC), and (G, H, I) *L. b. aranica* (white arrow indicates DAPI positive heterochromatin; 971 HCC).

been karyotyped, a chromosome set with many biarmed chromosomes has so far been observed only in *L. parva* (Gorman, 1969; Kupriyanova, 1980) and in *L. fraasii* (Odierna et al., 1995), and sex chromosomes similar to those of Pyrenean *Archaeolacerta* have been observed only in *L. vivipara* (Capriglione et al., 1994; Chevalier et al., 1979; Kupriyanova, 1990; Odierna et al., 1993). The peculiarity of the karyotype of the taxa from the Pyrenees is consistent with the morphological observations of Arribas (1993*a*) and Perez-Mellado et al. (1993), and further supports the hypothesis that these lizards belong to species distinct from *L. monticola*. In addition, the morphological results of Arribas (1994) on *L. aurelioi* and *L. bonnali bonnali* are further supported by karyological results, showing differences in chromosome number, morphology and heterochromatic material of the W-chromosome, behavior of the NOR-associated heterochromatin in the presence of Alu I (sensitive in *L. aurelioi*, resistant in *L. b. bonnali*), and centromeric heterochromatin content.

The specimen of *L. b. aranica* differ from those of the nominal subspecies in the number of biarmed chromosomes, as well as in some characteristics of heterochromatin. The difference in the diploid number appears fixed in all the populations of the two subspecies of *L. bonnali* (unpublished data). Fixation of Robertsonian mutations is known to be favored by

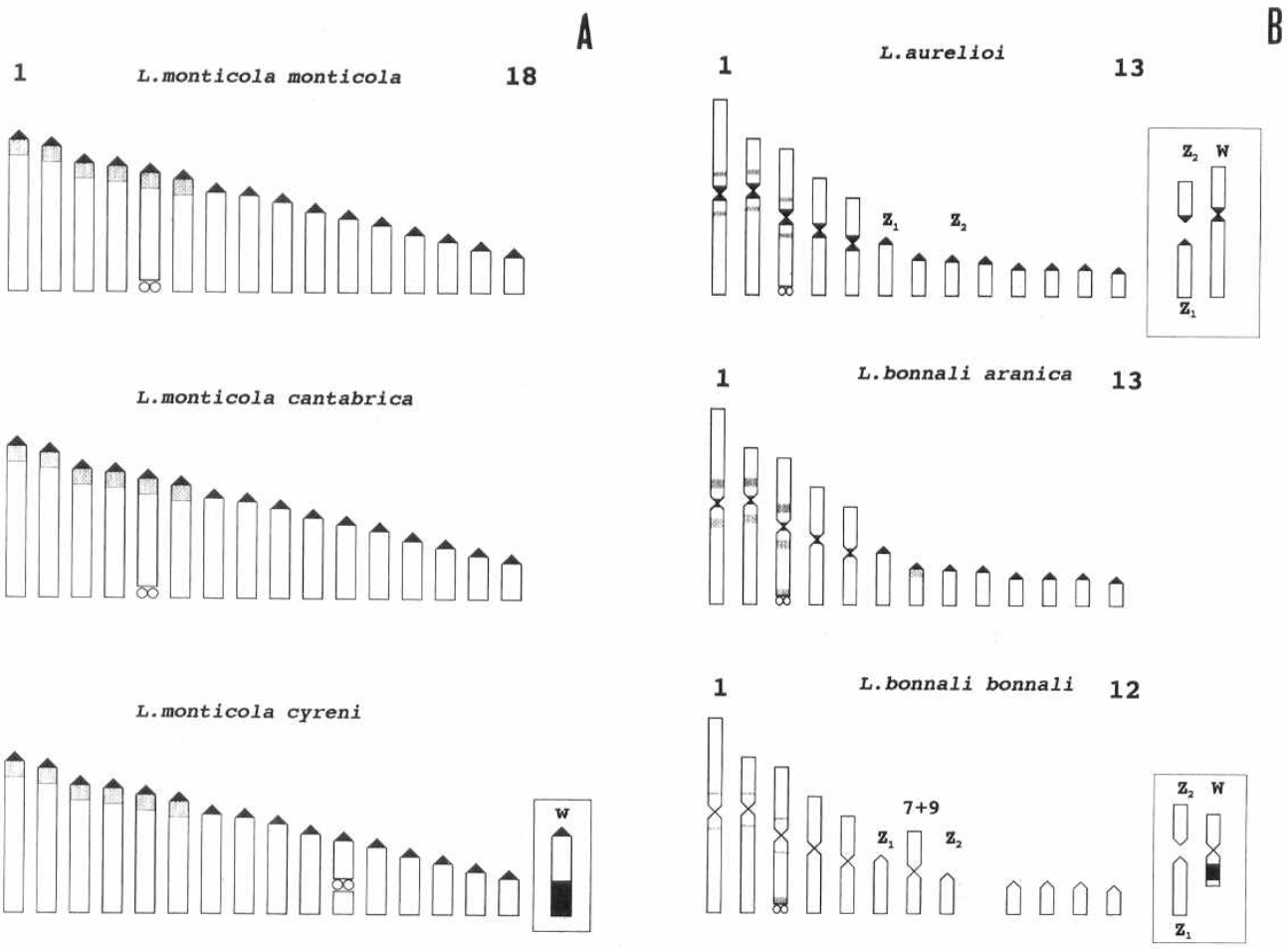

FIG. 6.—(A) Haploid idiograms of the three subspecies of *L. monticola*. (B) Haploid idiograms of *L. aurelioi* and two subspecies of *L. bonnali*. Solid black boxes, circles, and grey solid boxes show the C-, AgNOR, and Alu I banding patterns, respectively.

geographical isolation (Capanna et al., 1977), and this might also be true for *L. bonnali*. These high-mountain lizards live in isolated populations distributed along the various tops of the Pyrenees mountains. *Lacerta b. aranica*, in particular, shows a degree of morphological differentiation that, according to Arribas (1993*b*), may be indicative of an ancient isolation from the populations of the central Pyrenees. Thus, the karyological differences between *L. b. aranica* and *L. b. bonnali*, as well as the morphological (Arribas, 1993*b*) and the protein electrophoresis (Mayer, personal communication) ones, might be relevant and indicative of a higher rank than the subspecific one for the former.

Some comments should be made on the pattern shown by centromeric heterochromatin in the karyotypes of the various Iberian rock lizards. As shown in Figs. 2, 5, and 6A,B, in the karyotypes with all uniarmed chromosomes, intense centromeric C-bands are present in all the chromosomes, while in karyotypes with biarmed and uniarmed chromosomes only the latter possess evident centromeric C-bands; they are, instead, absent or very faint on the centromeres of the biarmed chromosomes. A similar situation has also been found in other organisms showing an intraspecific chromosome variability (Mayr et al., 1984; Redi et al., 1986); it has been suggested that variations in the amount and composition of the highly repetitive DNA sequences associated with constitutive heterochromatic blocks, especially centro-

meric, induce or hamper certain chromosome mutations like inversions and centric fusion (Capanna and Redi, 1994; Luke et al., 1992; Mayr et al., 1984; Redi et al., 1990). This may also be true for the Pyrenean rock lizards. In fact, it has been shown (Capriglione et al., 1989, 1991) that, in lizards, centromeric heterochromatin contains highly repetitive DNA sequences, some of which may be involved in centromere function (T. Capriglione, unpublished). Moreover it should be stressed that, in *L. b. aranica*, the 7th pair of acrocentric chromosomes possesses a DAPI-positive and Alu I-resistant large centromeric block of heterochromatin. This block is completely absent in *L. b. bonnali*, where the chromosomes of the 7th pair are fused with those of the 9th, forming a biarmed chromosome.

To conclude, our karyological study provides a genetic perspective that complements the morphological data on which a recent systematic decision of the Iberian rock lizards has been based. The karyological data confirm the morphological evidence that Iberian rock lizards are a heterogeneous group and that the Pyrenean species are clearly distinct from *L. monticola*; moreover, five distinct taxa of Iberian rock lizards have been identified karyologically (*L. monticola monticola* plus *L. m. cantabrica*, *L. m. cyreni*, *L. aurelioi*, *L. bonnali bonnali*, and *L. b. aranica*), and this suggests that Iberian rock lizards include more than the presently recognized three species. Further research on the possible role of certain highly repetitive DNA sequences in regulating this variability will allow us to begin understanding the evolving mechanisms responsible for the striking karyological variation found in these lizards.

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APPENDIX I

Specimens that were examined (all collected by O. Arribas). The specimens that were not destroyed during DNA extraction were deposited in the Herpetological Collection of V. Caputo, Ancona (HCC).

Lacerta monticola monticola.—PORTUGAL: 4 adult males (1101–1102 HCC) and 3 adult females (1103 HCC), locality A Torre, Sierra de Estrela.

Lacerta monticola cantabrica.—SPAIN: 3 adult males (1104 HCC) and 3 adult females (1105 HCC), locality Puerto de Vegarada, Leon.

Lacerta monticola cyreni.—SPAIN: 3 adult males (1099 HCC) and 2 adult females (1100 HCC), locality Puerto de Navacerrada, Madrid-Segovia.

Lacerta aurelioi.—SPAIN: 1 adult male (1116 HCC), Massif de Montroig, Lérida; 3 adult females (1106–1108 HCC), locality Estany de Barbote, Massif de la Pica d'Estats; 1 adult male (1109 HCC) and 1 adult female (1110 HCC), locality Estany de Sotllo, Massif de la Pica d'Estats, Lérida; 1 adult male (1112 HCC) and 2 adult females (1113–1114 HCC), locality Andorra.

Lacerta bonnali bonnali.—SPAIN: 2 adult males (1144–1145 HCC) and 3 adult females (1146–1148 HCC), locality La Estiba, Massif de Monte Perdido, Huesca.

Lacerta bonnali aranica.—SPAIN: 1 adult male (1118 HCC) and 2 adult females, locality Col de Barrados, Valle de Aran, Lérida.