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Sex chromosome evolution in reptiles: divergence between two lizards long regarded as sister species, *Lacerta vivipara* and *Lacerta andreanskyi*

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Abstract

Even if the common lizard *Lacerta vivipara* and endemic *Lacerta andreanskyi* from Moroccan Grand Atlas have the same mother species, the two species are definitely not closely related in present-day nature, where *L. vivipara* stands at variance from all other lacertids in terms of cytogenetics. The use of replication banding, C-bands and R-bands has allowed for the identification of all chromosome pairs and two sex chromosomes in *L. andreanskyi.* Its karyotype is typical of Lacertidae. The W chromosome, characterized by late replication, is nevertheless of a type previously unknown in the family. The comparison of Z and W chromosomes by different methods of chromosome banding indicates little homology between them, if any. The replication study has shown that there is no dosage compensation for the Z chromosome in (homogametic) males. That genetic inactivation precedes chromosomal mutations in non-vivipara lacertid evolution of the odd sex chromosome is suggested.

Introduction

More than thirty years have elapsed since J. M. van Brink (1959) published her *Expression morphologique de la digamétie chez les Sauropsidés et les Monotrèmes.* She concluded, after surveying nine species from the four major living reptilian taxa, that reptiles are not likely to have 'morphologically detectable heterochromosomes'. To-day, the number of chromosomally investigated species of reptiles has grown to be two orders of magnitude higher. Perhaps more importantly, cytogeneticists have a far less fixist view of a taxon's karyotypical features than in the 1950s. Among reptiles for example, the heterochromosome situation has turned out to be considerably more varied than in all other vertebrate classes. And at the species level, chromosomal polymorphism has proved rather commonplace in the animal kingdom. In addition, various cases of polytypism have been observed.

One of the five lizard species investigated for sex chromosomes by van Brink (1959) was *Lacerta (Zootoca) vivipara* or Common Lizard, a panpalearctic species. She observed 36 acrocentric chromosomes in both sexes of a population sampled in (pers. comm.) Valais, Switzerland. While Margot (1946), in further Switzerland populations, had found *L. vivipara* similarly homomorphic, Chevalier (1969) and Chevalier *et al.* (1979) found a Z_1Z_2W system, with the W a conspicuous metacentric chromosome, in populations from Massif Central, substantiating the observation of 35 chromosomes in females by Oguma (1935). It thus appeared that *L. vivipara* had some populations with and others without differentiated sex chromosomes, a situation raising the prospect of unraveling at least some circumstances of the genesis of chromosomal sex determination. So far, only differentiated sex chromosomes have been observed by our network, whether directly (Kupriyanova & Rudi, 1990; Volobouev, Salvidio & Pasteur in prep.; Volobouev *et al.* in prep.) or inferred from the absence of crossing over between

the sex factor and the known sex-linked gene (Goux & Pasteur, 1986; Salvidio *et al.*, 1990). But no populations from Switzerland and the Alps have been examined yet.

One possible parallel approach was to investigate a species which used to be ranged in the subgenus *Zootoca,* namely *Lacerta andreanskyi*, an endemic lizard from the Grand Atlas mountain range, Morocco. Was *L. vivipara* alone in bearing multiple sex chromosomes ($Z_1Z_1Z_2Z_2$ in males, Z_1Z_2W in females), one of them a large metacentric W, among lacertid lizards? More than fifty species of the family Lacertidae have now been cytogenetically examined by standard conventional staining: a majority possess karyotypes characterized by 36 acrocentric macrochromosomes, two dotlike microchromosomes, and absence of chromosomal heteromorphism (Gorman, 1973; Peccinini-Seale, 1981; Olmo *et al.*, 1987), whereas a minority of fourteen species display a sex chromosome system of the ZZ/ZW type, with the W being a microchromosome (Peccinini-Seale, 1981; Olmo *et al.*, 1987). However, the absence of visible chromosomal heteromorphism in conventionally stained chromosome preparations from most lacertid species should not be considered as absolute evidence for the lack of sex chromosomes. The existence of chromosome segments linked with sex determination has been demonstrated in the frog *Rana esculenta* (Schemp & Schmid, 1981) and the teleost *Poecilia sphenops* (Haaf & Schmid, 1984) by replication banding techniques; they are not distinguishable by any other methods in these species. A similar finding was recently made in the geckonid lizard *Lepidodactylus lugubris* (Volobouev & Pasteur, 1988). In the same way, a study of 13 species of lacertid lizards using the C-banding technique and 4'-6-diamidino-2-phenylindole (DAPI) staining revealed that in seven of them, sex chromosomes are similar in size and shape but the W chromosome is almost entirely heterochromatic (Olmo *et al.*, 1987).

No further data about chromosome banding in the family Lacertidae being available, we present here results on the DNA replication banding, C-bands and R-bands in chromosomes of *Lacerta andreanskyi.* These techniques have allowed us unambiguously to identify every autosomal pair and the sex chromosomes. While the *L. andreanskyi* karyotype is typical of a lacertid lizard, contrary to *L. vivipara*'s, its sex chromosomes nevertheless set a new type for the family.

Material and methods

The investigated animals, two males and two females of *Lacerta andreanskyi* (Nos. 1633-1634 and 1636-1637, respectively, in the collection of the Biogéographe et Ecologie des Vertébrés laboratory), were collected in the Oukaimeden vicinity, Grand Atlas, at 2500 and 2970 m.

Chromosome preparations were obtained from primary fibrolast cultures established from tail biopsies using the Wolf (1979) technique. Explants and a portion of the cells from our samples are routinely kept in liquid nitrogen in the cell and tissue collection of the Structure and Mutagenèse Chromosomiques laboratory.

Mitotic chromosomes have been studied with RGH R-banding and CBG C-banding (see ISCN, 1985) after, respectively, Carpentier *et al.* (1972) and Sumner (1972). The replication banding (RBG) has been studied following the method of Viegas-Péquignot and Dutrillaux (1978); RBG pattern has been induced by bromodeoxyuridine (BrdU) incorporation, after synchronization of the cultures by thymidine. At least 10 metaphase and 10 prometaphase plates have been analysed for each specimen.

Results

The karyotype of *Lacerta andreanskyi* is composed of 38 chromosomes, with 18 pairs of acrocentric macrochromosomes one of which is the sex chromosomes, and one pair of microchromosomes.

R-banding (Fig. 1) shows that, besides autosomal sets identical in both sexes, the female sex chromosomes consist of a subtelocentric Z chromosome similar in size to autosomes 13-14 and a subtelocentric W similar in size to autosome 17. Males possess two Z chromosomes.

C-banding (Fig. 2) revealed the presence of C-positive heterochromatin in all macrochromosome pairs, but with some differences in extent between the pairs. The W chromosome is extensively C-positive in

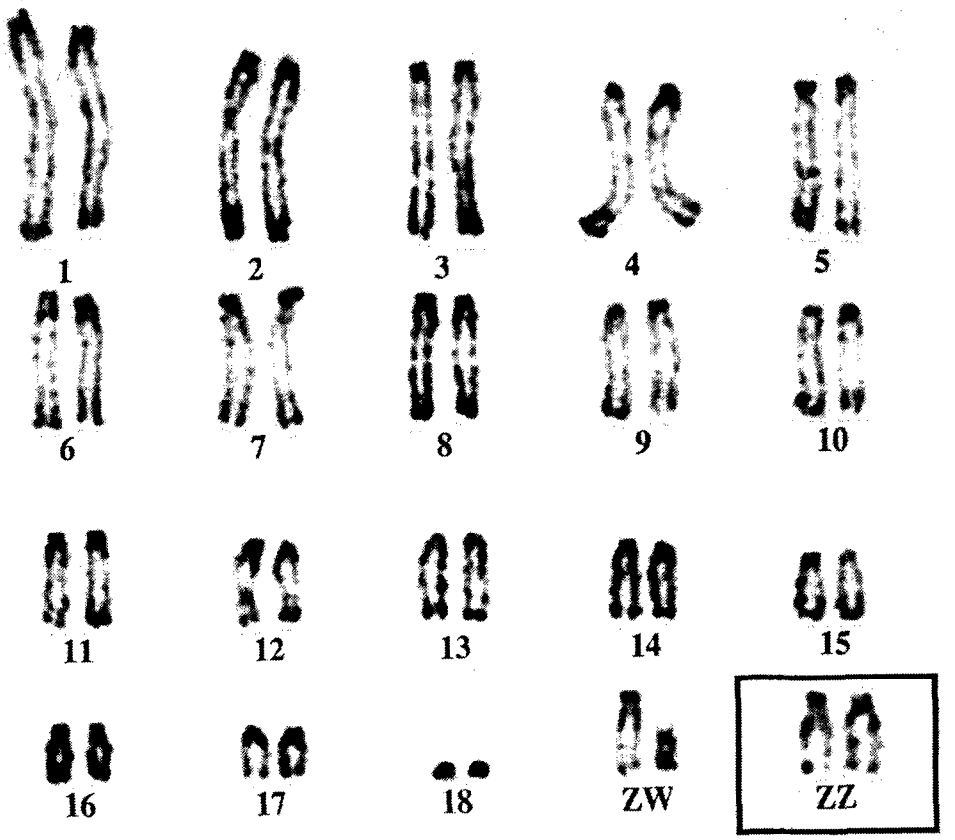

Fig. 1. R-banded (RHG) karyotype of a female *Lacerta andreanskyi*, with male sex chromosomes (ZZ) in inset.

both distal and proximal regions, and these are separated by a very narrow euchromatic region. The Z chromosomes have small pericentromeric and telomeric blocks of C-heterochromatin, and the short arms of the two sex chromosomes are heterochromatic.

Replication banding (Figs. 3 and 4): All pairs of autosomes and both sex chromosomes have been identified precisely. The W chromosome is late replicating, as can be inferred from its very pale appearance, except for the two thin bands on Figure 3; these correspond to the narrow euchromatic region indicated on Figure 2. The Z chromosomes replicate their DNA together with the autosomes in both sexes.

All techniques point to a thorough dissimilarity between the W and Z chromosomes. It may be that an accumulation of heterochromatin on the original W was accompanied by rearrangements that profoundly changed its structure. If homologous segments exist, they should be restricted to the narrow non-heterochromatic region separating the proximal and distal blocks of C-positive heterochromatin in chromosome W. As disclosed by replication banding, this region includes two early-replicating bands discernible at metaphase.

The fact that the narrow euchromatic region of the W has proved visually detectable through two techniques indicates that its DNA sequences may still include several kilobases.

Discussion

Reasons why the exotic and very rare *L. andreanskyi* has been ranged together with *L. vivipara*, the commonest lizard in the world and a largely northern species, were overall morphological resemblance and the restriction of *L. andreanskyi* to high altitude in its warm country, as this seemed to match the low average temperature of *L. vivipara* habitats (see a review in Arnold, 1989).

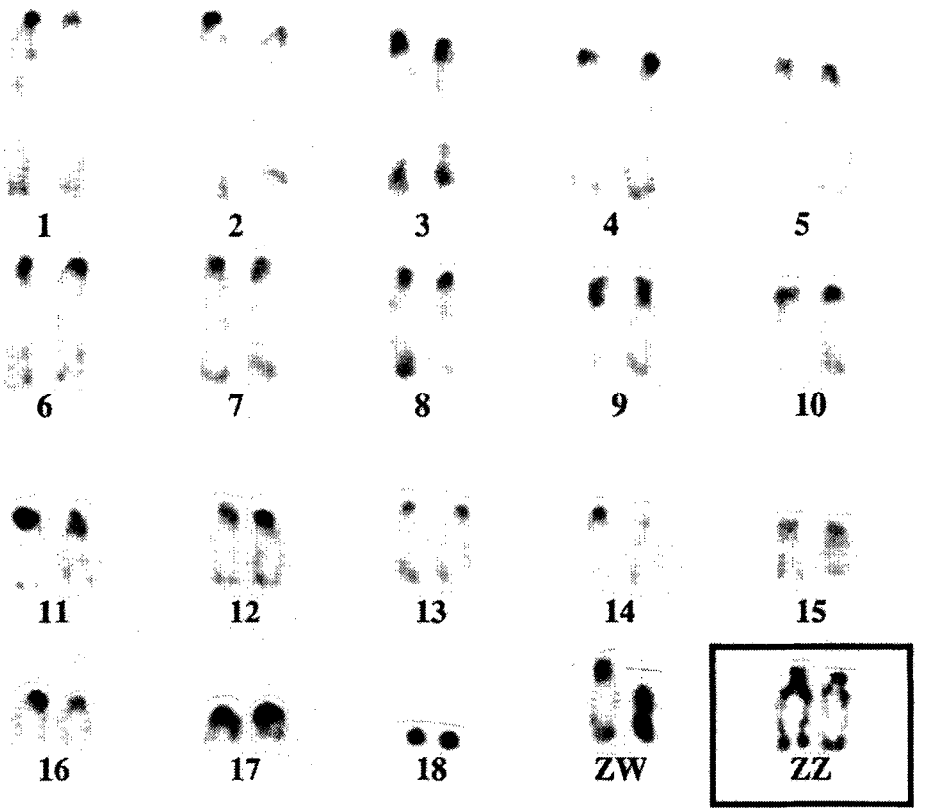

Fig. 2. C-banded (CBG) karyotype of a female *L. andreanskyi,* with male sex chromosomes (ZZ) in inset.

The present study shows that, even if the two species had the same mother species, they have so much diverged by now that *L. andreanskyi* will be of little relevance for an understanding of the evolution of *L. vivipara,* a species of prime interest for an evolutionary study of sex determination. The *L. andreanskyi* karyotype, contrary to that of *L. vivipara,* displays a typical lacertid pattern with 38 chromosomes including one pair of microchromosomes. Minor differences with previous lacertid data, such as the presence of subtelocentric chromosomes, might be explained by the lesser degree of chromosome spiralization on preparations obtained from long-term cell cultures, knowing that previous descriptions of lacertid karyotypes were based on direct preparations. With its double sex chromosome system and its large, essentially euchromatic W associated with absence of microchromosomes (Chevalier *et al.,* 1979; our unpublished data), *L. vivipara* stands alone indeed among Lacertidae in terms of cytogenetics, as it does (see Pilorge, 1984) ecophysiologically.

The applied techniques permit to distinguish all autosomal pairs of *L. andreanskyi* and provide unequivocal identification of its W chromosome and its Z chromosome. Since the W chromosome is similar in size to the smallest pair of acrocentrics, it represents an intermediate stage between the two kinds of W chromosomes known until now in Lacertidae (*vivipara* excluded), i.e. macrochromosomal acrocentrics and microchromosomes (see Olmo *et al.,* 1987). However, by its C-banding pattern the *andreanskyi* W is comparable to the W chromosomes observed in some previously studied Lacertidae (see Fig. 4 of Olmo *et al.,* 1987).

The absence of dosage compensation in male Z chromosomes, an unexplained phenomenon, has not met with any exception we are aware of in organisms with female heterogamety (birds, lepidoptera, ZW reptilia). *L. andreanskyi* is no exception either, since we have found the two Z chromosomes in synchrony with

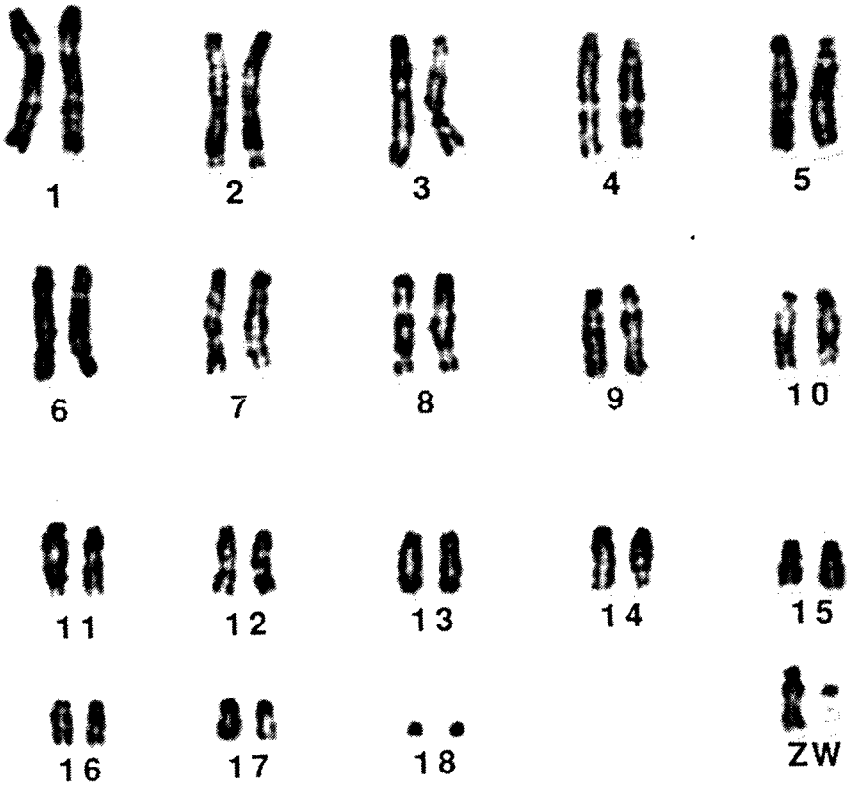

Fig. 3. R-banded (RBG) prometaphase chromosomes of a female *L. andreanskyi.*

autosomes in all metaphases of males.

Regarding the origin and evolution of sex chromosomes in reptiles, Singh *et al.* (1976, 1979) and Jones and Singh (1985) put forward a hypothesis based on molecular and chromosomal banding studies of snakes. The first step in sex chromosome differentiation would be the accumulation, in one of the non-specialized homologues, of specific satellite DNA leading to genetic inactivation of the concerned chromosome. The structural rearrangements could occur later, irrespective of when the inactivation is completed. The Mullerian model of sex chromosome evolution endorsed by Ohno (1967, 1979), on the other hand, assumes that structural rearrangements take place first, and gene inactivation would be a consequence of the ensuing isolation of the two sex chromosomes through suppression of crossing over.

The sex chromosomes of *Lacerta vivipara* clearly exemplify the Ohno model (see Salvidio *et al.*, 1990). But they are thoroughly atypical among Lacertidae. Concerning other species in the family, it is hard, in absence of molecular data, of C heterochromatin patterns and of known sex-linked genes, to conclude in favor of either of the two models. Nevertheless, four facts suggest the Singh and Jones model as a better working hypothesis.

First, karyotype patterns rule out the occurrence of various mutation types as triggers of inactivation: robertsonian translocation, pericentric inversion, important duplication. Secondly, the evolutionary stage which we see in the *andreanskyi* W chromosome suggests a progressive evolution from a homomorphic chromosome pair carrying the sex factor. Thirdly, this stage is quite rare among lacertids. It follows that the intermediate condition between the macro- and microchromosomal states of W chromosomes is transient, relatively short-lasting in these lizards. (The minute range and restricted niche of *L. andreanskyi* might be related to this, if it indicates a phase of adaptive disequilibrium.) Fourthly, uniformity in heterogametic sex and in W microchromosomes among heteromorphic lacertids, and karyotypic uniformity among homomorphic lacertids some of which, if not all, necessarily carry sex factors, strongly

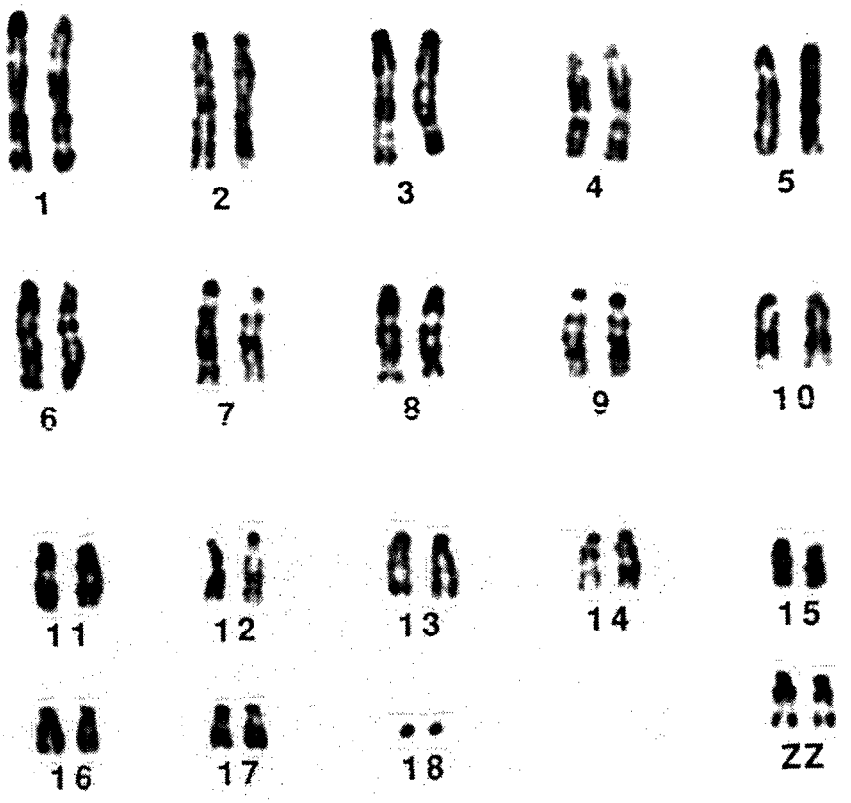

Fig. 4. R-banded (RBG) prometaphase chromosomes of a male *L. andreanskyi*. Careful examination of centromeric regions in the four figures shows that most chromosomes could have second arms, so short that they cannot be clearly distinguished in every preparation.

suggest uniformity in sex chromosome events in all lineages.

These four observations do not agree well with chromosomal mutations being the leading mechanism of sex chromosome differentiation in non-vivipara Lacertidae. The first two observations because they do not suggest a sudden morphological change. The third because it would require an unusually high rate of gene rearrangements. The fourth because the haphazardness of chromosomal mutations is incompatible with it.

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