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Evolution of sex-chromosomes in lacertid lizards

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Abstract. The occurrence and form of sex chromosomes were investigated with the aid of C-banding and 4′-6-diamidino-2-phenylindole (DAPI) staining in 13 species of lacertid lizards. The results obtained show the presence in five species of a female heterogamety in which the two sex chromosomes have the same shape and size, but the W differs from the Z in being almost entirely heterochromatic. This condition is clearly similar to that found in some snakes and considered to be an early stage of differentiation of sex chromosomes by Singh et al. (1976, 1980). A more evolved condition may be that found in three other species in which the W is distinctly smaller than the Z. A third situation is that found in all *Podarcis* species which, even though they are considered to be among the more evolved species in the family, possess two sex chromosomes that are indistinguishable. In general, the situation in lacertids may be compatible with the hypothesis of sex chromosome evolution put forward by Singh et al. (1976, 1980). However a differentiation mechanism of this kind does not seem to be well established in lacertids, and is probably not the only mechanism that is in operation in this family.

Introduction

Reptiles, and in particular lizards and snakes, are interesting with regard to the evolution of sex chromosomes. Singh et al. (1976, 1980) have described various levels of differentiation of sex chromosomes in snakes. Sex chromosomes have been identified in several families of lizards (Olmo 1986). These chromosomes show considerable inter- and intraspecific variability and seem to have originated through different primary mechanisms of differentiation. Singh et al. (1980) considered that one of the primary mechanisms of sex chromosome differentiation is the accumulation on one member of a chromosome pair of a specific highly repeated (satellite) DNA sequence, accompanied by the appearance of heterochromatin in that chromosome. In two species of lacertid lizards, *Gallotia galloti* and *Takydromus sexlineatus* we have identified, by Giemsa C-banding, sex chromosomes that show various analogies with those that have been described in snakes as intermediate in their differentiation (Olmo et al. 1984, 1986). We have now extended our studies in lacertid lizards by applying to a wider range of species the C-banding technique and a chromosome banding technique based on the use of 4′-6-diamidino-2-phenylindole (DAPI), a fluorochrome that is relatively specific for A + T-rich DNA (Schweizer 1980).

Materials and methods

The occurrence and form of sex chromosomes were investigated with the aid of Giemsa + DAPI staining in 13 species of lacertids: *Acanthodactylus erythrurus, G. galloti, Lacerta dugesii, L. lepida, L. monticola, L. viridis, Meroles cuneirostris, Podarcis melisellensis, P. sicula, P. tiliguerta, P. wagleriana, Psammodromus algirus* and *T. sexlineatus.*

All the specimens of *A. erythrurus, L. monticola* and *Psammodromus algirus* and some specimens of *L. lepida* were kindly provided by Mr. V. Caputo. *A. erythrurus* was collected at Sierra de Gregos near Madrid, *L. monticola* was collected at Albufera near Valencia (Spain), and *P. algirus* was collected near Taza (Morocco). The specimens of *L. lepida* were collected at Molina de Aragon near Saragoza (Spain). Other specimens of this species were purchased from an animal dealer (Drs. W. De Rover); they came from a different region of Spain, but the exact locality in which they were collected is unknown. Specimens of *M. cuneirostris* were kindly provided by Dr. W. Mayer and were collected near Luderitz, Rosh Pinah and Aus (South West Africa). Specimens of *P. tiliguerta* were collected on the island of La Maddalena (Sassari, Italy) and kindly provided by Dr. S. Casu. Specimens of *P. wagleriana* were collected near Porto Palo (Siracusa, Italy) and near Primo Sole (Catania, Italy) and kindly provided by Dr. M. Capula. Specimens of *P. sicula* were collected by us in various parts of the Campania region (Italy). Other species were obtained from the animal supplier Drs. W. De Rover (Holland) and the precise localities in which they were collected are unknown.

All animals were injected intraperitoneally with phytohaemagglutinin (Phytohaemagglutinin M, Difco, 6.7% in distilled water; 0.02 ml/g body weight) and colchicine (0.5 mg/ml; 0.01 ml/g body weight). After 45 min they were fully anaesthetized with "MS 222" (Tricainemetasulphonate) and dissected to obtain intestine, bone marrow and testes for chromosome preparations.

Mitotic metaphase preparations were obtained by methods described previously (Odierna et al. 1985; Olmo et al. 1986) involving spreading or scraping followed by air drying. Chromosomes were stained by the Giemsa C-banding method described by Sumner (1972) with suitable modifications (Odierna et al. 1985; Olmo et al. 1986) and by a method that combines treatment with a saturated solution of

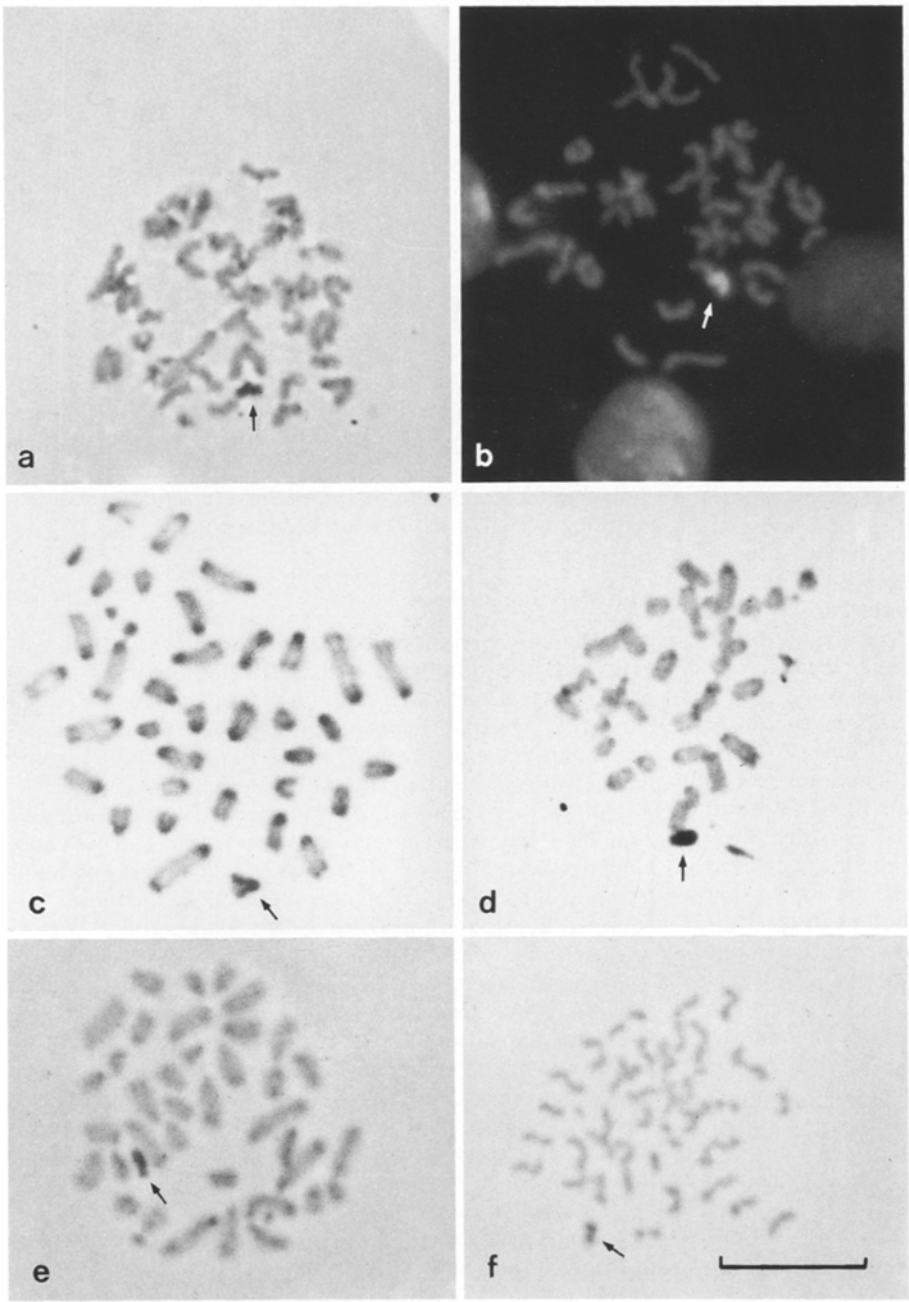

Fig. 1 a–f. Metaphase plates of: **a, b** *Takydromus sexlineatus*, **c** *Meroles cuneirostris*, **d** *Acanthodactylus erythrurus*, **e** *Gallotia galloti*, **f** *Psammodromus algirus*. **a, c, d, e,** and **f** were stained by the C-banding method; **b** was stained with DAPI. *Arrows* indicate the W chromosomes. Bar represents 10 μm

$Ba(OH)_2$, under the same conditions as used for C-banding, followed by staining for 20 min in DAPI (0.6 μg/ml in McIlvaine's buffer, pH 7).

Results

C-banded somatic metaphases of each of the species investigated are shown in Figures 1, 2 and 3. It is evident that, as already described for *G. galloti* and *T. sexlineatus* (Olmo et al. 1984, 1986), another four species belonging to the same family, *A. erythrurus*, *L. monticola*, *M. cuneirostris* and *P. algirus*, show female heterogamety. In all four species the two sex chromosomes are of the same size and shape. However the W chromosome differs from the Z chromosome in being almost entirely heterochromatic. In *L. lepida* we found intraspecific variability: the specimens coming from Molina de Aragon possess sex chromosomes with the W homomorphic heterochromatic as in the above-mentioned species (we call this *L. lepida* type I); the other specimens show instead heteromorphic sex chromosomes in which the W is a microchromosome (we call this *L. lepida* type II). The W chromosomes of *G. galloti*, *L. monticola*, *L. lepida* type I, *M. cuneirostris*, *P. algirus* and *T. sexlineatus* each have a small interstitial region of euchromatin (Fig. 4). In *A. erythrurus* a similar region is present near the centromere of the W. The heterochromatin of the W chromosome is strongly DAPI positive (Fig. 1b).

Evidence of sex chromosome heteromorphism was also found in female interphase nuclei of *A. erythrurus*, *G. galloti*, *L. lepida* type I, *L. monticola*, *M. cuneirostris* and *P. algirus* in the form of a single conspicuous Giemsa-positive body (Fig. 5). This body is also stongly DAPI positive and

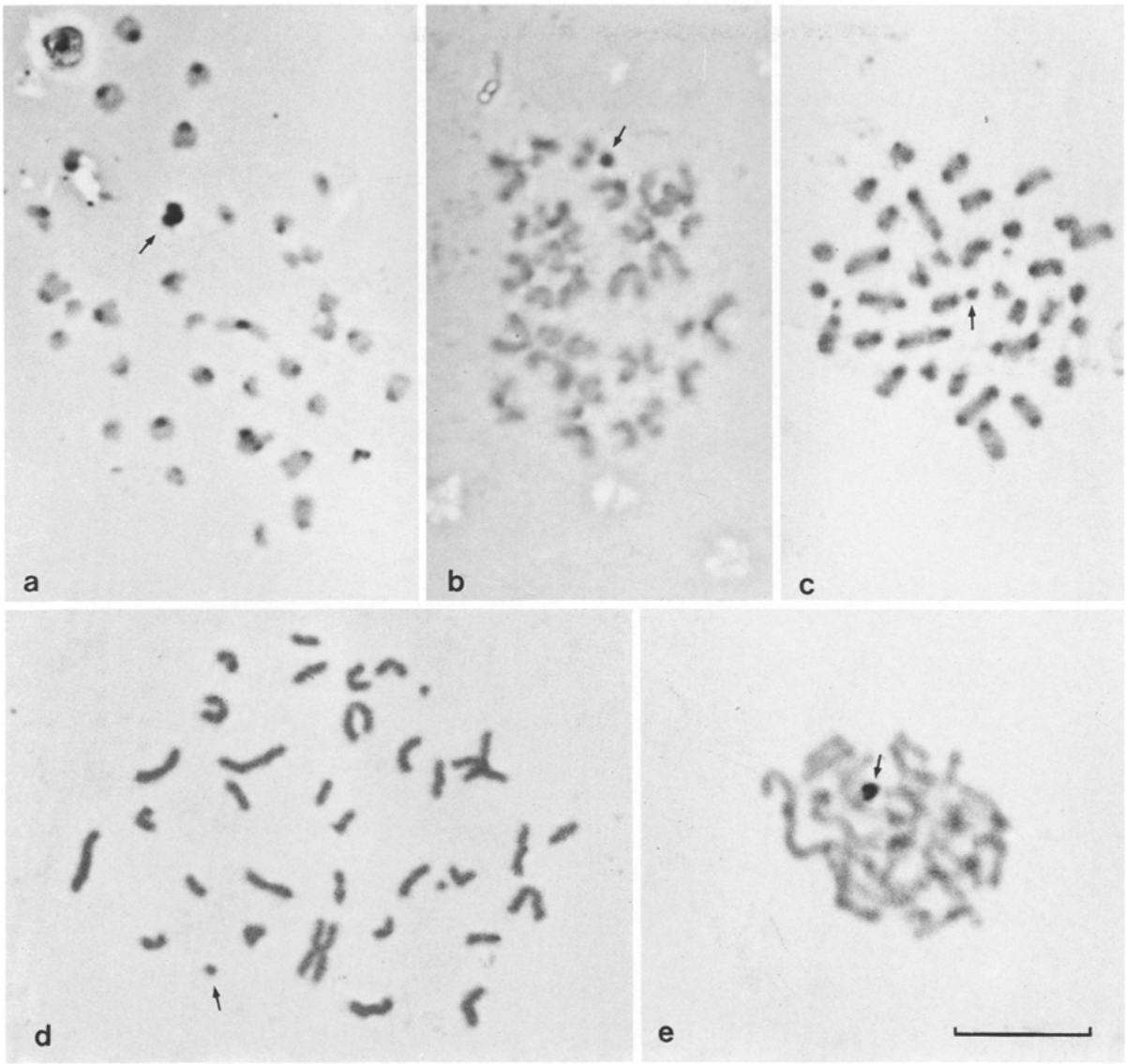

Fig. 2a–e. Metaphase plates stained by the C-banding method: **a** *Lacerta monticola*, **b** *L. viridis*, **c** *L. dugesii* **d, e** *L. lepida*. *Arrows* indicate the W chromosomes. Bar represents 10 μm

probably represents the condensed heterochromatin of the W chromosome (Fig. 5). No such body is found in interphase nuclei from *T. sexlineatus* (Fig. 5).

A different situation exists in *L. dugesii* and *L. viridis* (Fig. 2). These two species show female heterogamety of the ZW type in which the W is not only heterochromatic but is also smaller than the Z. In *L. viridis* the W is intermediate in size between the smallest macrochromosome and the microchromosomes. In *L. dugesii* as in *L. lepida* type II the W is comparable in size to a microchromosome.

The methods that we employed revealed no differentiated sex chromosomes in any species of *Podarcis* (Fig. 3).

Discussion

Table 1 summarizes current information on the incidence of sex chromosomes in lacertid lizards. At least four different situations can be distinguished: (1) sex bivalents that are wholly euchromatic; (2) sex bivalents in which the Z is euchromatic and the W is heterochromatic; (3) a condition in which the W is distinctly smaller than the Z, and (4) a Z_1Z_2W situation such as is found in *L. vivipara* in which the W is a biarmed macrochromosome.

The sex bivalents that are present in *A. erythrurus*, *G. galloti*, *L. lepida* type I, *L. monticola*, *M. cuneirostris* and *T. sexlineatus* are clearly similar to those found in some colubrids and accordingly they may be judged to be at an early stage in their differentiation (Singh et al. 1976, 1980). As in snakes, these five lacertids possess sex homologues that are homomorphic but the W differs from the Z in being heterochromatic and C-banding positive.

A similarity in composition could correspond to this morphological resemblance. The lacertids that we have studied have W chromosomes that stain diffusely and intensely with the fluorochrome DAPI, specific for DNA that is rich in A+T (Schweizer 1980). It is well known that the W chromosomes of snakes are rich in certain sex-specific satellite DNAs, such as the satellites III and IV of *Elaphe radiata*, and the Bkm sequence of *Bungarus ceruleus*, both of which are rich in A+T (Singh et al. 1976, 1980, 1984).

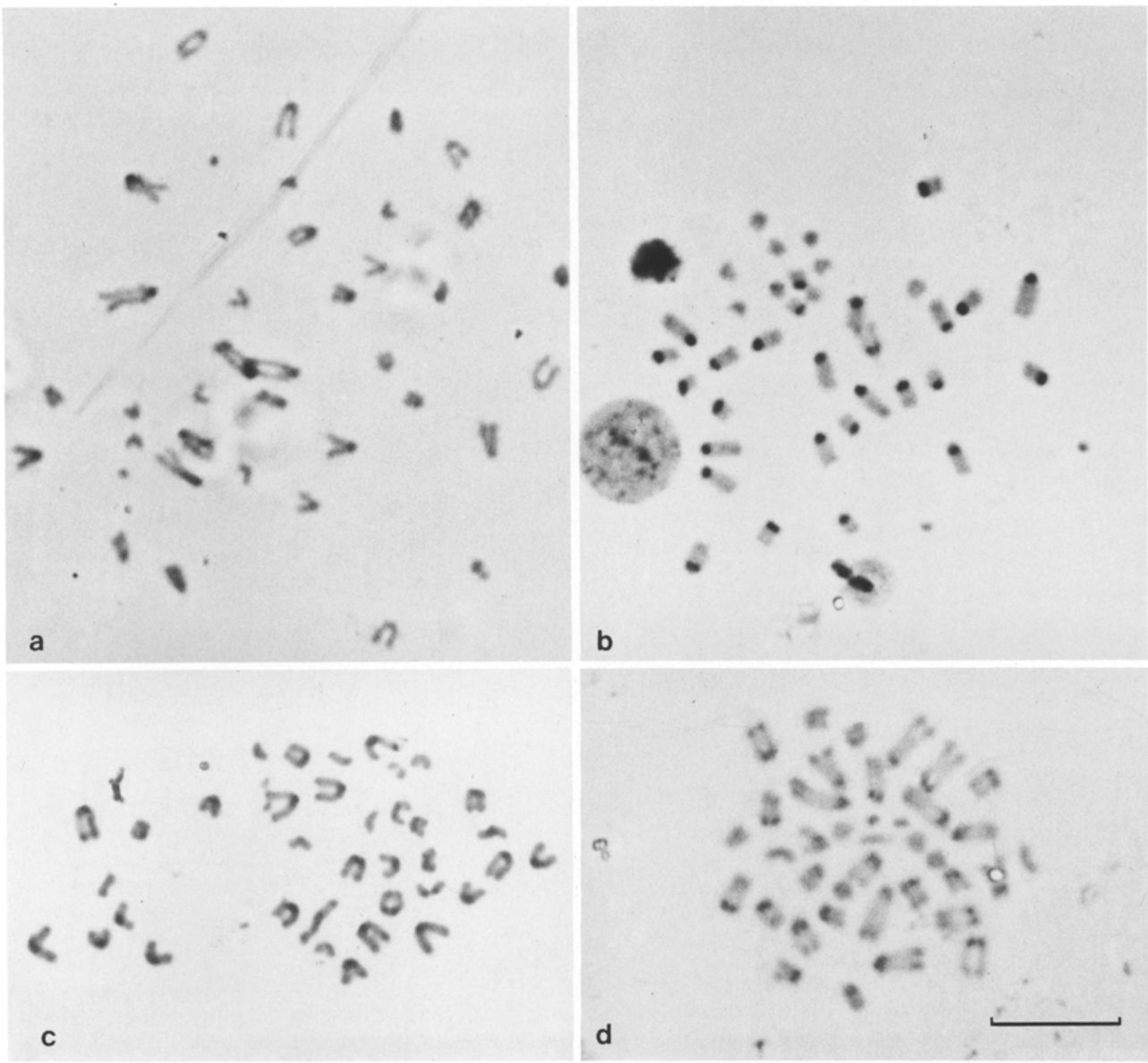

Fig. 3a–d. Metaphase plates stained by the C-banding method of various species of *Podarcis:* **a** *P. melisellensis*, **b** *P. sicula*, **c** *P. tiliguerta* and **d** *P. wagleriana*. Bar represents 10 μm

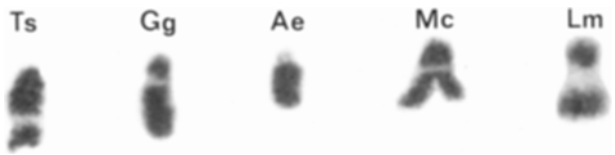

Fig. 4. Homomorphic heterochromatic W chromosomes of: *Ts Takydromus sexlineatus, Gg Gallotia galloti, Ae Acanthodactylus erythrurus, Mc Meroles cuneirostris, Lm Lacerta monticola.* Note the presence of a small euchromatic region present at different-levels on the W chromosome

As in snakes, the homomorphic sex bivalents of lacertids, including the euchromatic Z and the heterochromatic W may represent a primitive state in the differentiation of sex chromosomes. This view is upheld by their phyletic distribution. They have been found in various species, some quite distantly related from the evolutionary standpoint, and in particular they have been found in genera such as *Takydromus* which separated very early from other lacertids (Arnold 1984), and *Gallotia* which is considered to be one of the oldest members of the family (Lopez-Jurado et al. 1986). Starting from the presumed primitive condition, euchromatic Z and heterochromatic W, the subsequent evolution of sex chromosomes in lacertids would have proceeded by a progressive reduction in the size of the W chromosome, leading to the condition that is found in *L. viridis*, where the W chromosome is intermediate in size between macro- and microchromosomes. The end-point in this process would be such as is found in *L. dugesii* and *L. lepida* type II, and other lacertids in which the W chromosome is comparable in size with the microchromosomes. This transition could have happened independently in different species, since in *L. lepida* we found specimens having homomorphic sex chromosomes with the W heterochromatic, and specimens, probably belonging to a different population, in

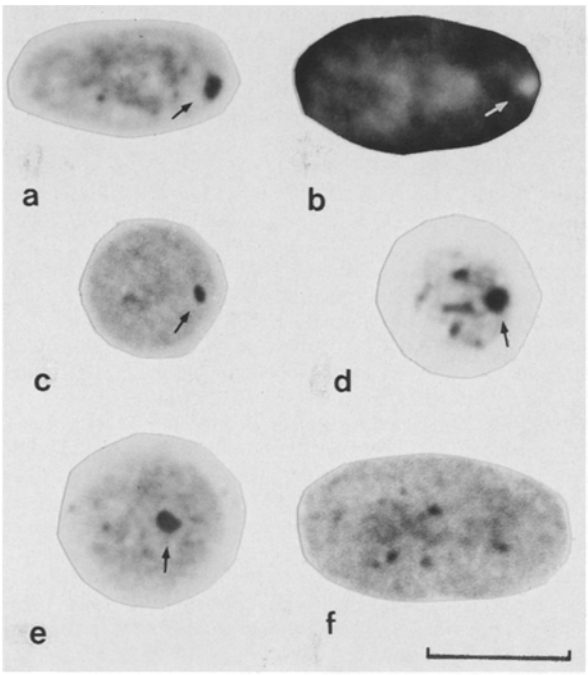

Fig. 5a–f. Interphase nuclei of: **a, b** *Acanthodactylus erythrurus,* **c** *Gallotia galloti,* **d** *Lacerta monticola,* **e** *Meroles cuneirostris,* **f** *Takydromus sexlineatus.* Note the presence of a Giemsa-positive heterochromatic body in the various species except for *T. sexlineatus* (*arrows*). This body is DAPI positive (**b**). Bar represents 10 μm

which the W was heteromorphic. A similar situation may be present also in other species, like *L. viridis, L. vivipara* and *P. algirus* (Chevalier et al. 1979; De Smet 1981; Kupriyanova 1987, personal communication). Intraspecific variations in sex chromosome morphology have been reported in several other reptiles (Olmo 1986).

The situation in various species of *Podarcis* deserves special attention as sex bivalents are not distinguishable in this genus either by their morphology or their heterochromatin content. Two matters are worth mentioning in this regard. First, *Podarcis* is one of the more highly evolved genera of the family (Arnold 1973). Second, De Smet (1981) has identified some heteromorphic sex chromosomes in *P. melisellensis* and *P. sicula* where the W chromosome is a microchromosome.

One possible explanation of the *Podarcis* situation is that in each species there coexists, perhaps in different populations, different levels of sex chromosome differentiation: one in which the sex bivalents are indistinguishable, one in which the Z is euchromatic and the W heterochromatic and one in which the two chromosomes are of different sizes. A second possibility is that in *Podarcis* a process of differentiation may have occurred other than the accumulation of heterochromatin. In this connection it may be appropriate to mention the situation seen in the gekkonids *Gehyra* and *Heteronotia* where differentiation of the sex chromosomes has happened not through accumulation of heterochromatin but on account of paracentric inversions (Moritz 1984a, b). A paracentric inversion that did not

Table 1. Current information on the incidence of sex chromosomes in lacertid lizards

Species	Sex chromosome morphology	References
Acanthodactylus erythrurus	Hom. Het.	This paper
Eremias arguta	Micro	Ivanov and Fedorova (1973)
E. olivieri	Micro	Gorman (1969)
E. velox	Micro	Ivanov et al. (1973)
Gallotia galloti	Hom. Het.	Olmo et al. (1986)
Lacerta agilis	Micro	De Smet (1981)
L. armeniaca	Micro	Darevsky et al. (1978)
L. dugesii	Micro	This paper
L. lepida	Micro	Olmo et al. (1986)
L. lepida	Hom. Het.	This paper
L. monticola	Hom. Het.	This paper
L. strigata	Micro	Ivanov and Fedorova (1970)
L. trilineata	Micro	Gorman (1969)
L. viridis	S. macro	Olmo et al. (1986)
L. viridis	Micro	De Smet (1981)
L. viridis	Hom.?	Chevalier et al. (1979)
L. vivipara	Biarmed	Chevalier (1969)
L. vivipara	Hom.?	L. Kupriyanova (1987), personal communication
Meroles cuneirostris	Hom. Het.	This paper
Ophisops elegans	Micro	Bhatnagar and Yoniss (1976)
Podarcis melisellensis	Micro	De Smet (1981)
P. melisellensis	Hom. Eu.	This paper
P. sicula	Micro	De Smet (1981)
P. sicula	Hom. Eu.	This paper
P. tiliguerta	Hom. Eu.	This paper
P. wagleriana	Hom. Eu.	This paper
Psammodromus algirus	Micro	De Smet (1981)
Psammodromus algirus	Hom. Het.	This paper
Takydromus sexlineatus	Hom. Het.	Olmo et al. (1984, 1986)

Hom, homomorphic; *Het.*, W completely heterochromatic; *Eu*, W euchromatic; *S. macr*, W intermediate in size between the smallest macro and the microchromosomes; *Micro*, W comparable in size to a microchromosome; *Biarmed*, W biarmed macrochromosome; *?*, the C-banding of the W chromosome is not known

include regions near the centromere could not have been detected by our banding techniques in the species that we have studied, since all the chromosome C-bands that we have identified are centromeric or closely pericentric (Olmo et al. 1986). Yet another possibility is a secondary dedifferentiation of the sex chromosomes brought about by a loss or drastic reduction in the amount of sex-specific satellite DNA sequences. However, no examples of such a phenomenon are known, and in any case it would not explain the cases of heteromorphic sex chromosomes described by De Smet (1981).

In general, the situation seen in lacertids may be compatible with the hypothesis of Singh et al. (1976, 1980) in so far as the first step in the differentiation of sex chromosomes may be the accumulation on one or other of the homologues of a specific highly repetitive DNA accompanied by an increase in heterochromatin, these events preceding any structural or morphological rearrangements. However a differentitation mechanism of this kind does not seem to be well established in lacertids, and is probably not the only mechanism that is in operation. In this context four points are of special significance. (1) *L. vivipara* is clearly distinct from other species with regard to the differentiation of its sex chromosomes. However, since the C-banding pattern of the chromosomes of this species is not known we cannot exclude the possibility that the sex chromosomes of *L. vivipara* have differentiated from a primitive state similar to that found in *Takydromus* and *Gallotia*. (2) Intraspecific variability in sex chromosome morphology has been found in various species of lacertids. (3) The pattern of distribution of heterochromatin differs from species to species, at least with regard to the species that we have investigated. (4) The accumulation of heterochromatin has not resulted in complete "inactivation" of the W chromosome in all species. Indeed in interphase nuclei of *T. sexlineatus* the W chromosome seems to be mainly euchromatic and therefore supposedly active in transcription.

A hypothesis that could provide the best explanation of our observations in lacertids is that in lizards and perhaps in some other reptiles sex chromosome differentiation is a process that has taken place repeatedly and independently and through a variety of mechanisms in different taxa (Mengden 1981; Moritz 1984a, b; Olmo 1986). In any event, the diverse situations that we see in lacertids render the group particularly favourable for studies of the evolution and differentiation of sex chromosomes both from the cytological and molecular standpoints.

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