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# Further data on sex chromosomes of Lacertidae and a hypothesis on their evolutionary trend

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Abstract. Sex chromosomes were studied in eight species of lacertid lizards using C-banding, G-banding and restriction enzyme treatment. All of the species showed female heterogamety. The W chromosome was a microchromosome in *Lacerta graeca* and *Ophisops elegans*. Two types of W were found in *Lacerta vivipara*; in specimens from The Netherlands it was metacentric, whereas in specimens from Russia it was acrocentric or subtelocentric. The W chromosome was homomorphic or nearly homomorphic but completely C-banded and heterochromatic in *Lacerta agilis, Podarcis hispanica, Algyroides moreoticus* and *A. nigropunctatus*. In was only possible to find sex chromosomes using the G-banding method in *Podareis sicula*. The results obtained, together with data in the literature, suggest that sex chromosomes are likely to be present in all Lacertidae and that their differentiation took place repeatedly and independently in different taxa within the family. A model for sex chromosome evolution in the family, in which the starting point was the heterochromatization of the W chromosome, is proposed.

## Introduction

The studies carried out so far on the sex chromosomes of lacertid lizards have shown an extremely heterogeneous situation. Various species have either homomorphic or highly differentiated sex chromosomes, and there are also species in which they seem to be completely lacking (Olmo et al., 1987). In addition, the occurrence of specimens of the same species with sex chromosomes at different stages of differentiation has suggested that sex-chromosome differentiation in this family took place repeatedly and independently through a variety of mechanisms in the different taxa (Olmo et al., 1987).

These observations led us to try to obtain a deeper insight into the sex chromosomes of these lizards. The aim of the present paper is to assess the occurrence and the differentiation level of sex chromosomes in various species of lacertid lizard, as well as to determine the possible mechanism(s) involved in sex chromosome differentiation within the family.

## Material and methods

Sex chromosomes were studied in eight species of Lacertidae:
*Algyroides moreoticus*; two females and one male captured in the Peloponnese, Greece.
*A. nigropunctatus*; three females and one male captured on the island of Krk, Yugoslavia. These were a gift from H. in den Bosch.
*Lacerta agilis* and *L. vivipara*; two males and two females of both species from Hatertse en Overasselte Vennen, The Netherlands. These were a gift from H. Strijbosch. Three males and four female *L. vivipara* were obtained from the neighbourhood of Pskov, Russia. *L. graeca*; two males and three females were collected in the Peloponnese, Greece. These were a gift from Dr. Mayer.
*Ophisops elegans*; two females from the neighbourhood of Uzunkopru, European Turkey. These were a gift from Ing. Capolongo.

All the specimens of the various species were deposited in the personal herpetological collection at Naples of V. Caputo.

Mitotic plates and staining by the C-banding technique were prepared according to methods previously described (Olmo et al., 1987). G-banding was performed according to Seabright's method modified by Odierna and Olmo (1988). Chromosomes were exposed to trypsin (Difco 1:250, 1 ml of the stock solution in 100 ml water) for 1 min, washed in 95% alcohol, air dried, incubated in 2 × SSC at 70°C and stained in 5% Giemsa in buffer at pH 7 for 10 min. Treatment with Alu I restriction enzyme (which usually produces a C-like banding, Lopez-Fernandez et al., 1991) was carried out following Miller et al. (1984), by exposing metaphasic plates to Alu I (40 units diluted in 100 μl specific probe mixture) for 12 h. Control slides received only the probe mixture.

## Results

The various methods employed in our investigation showed sex chromosomes in all the species studied. Previous observations were confirmed, and, in addition, differentiated sex chromosomes were detected in species in which they were supposed to be absent.

All the species showed female heterogamety. In some species the W chromosome was markedly different from the Z chromosome in shape and size. In *Lacerta graeca* and *Ophisops elegans* the W chromosome was a microchromosome, and was a little larger-sized than the two microautosome (fig. 1). In the preparations treated with C-banding and the restriction enzyme Alu I, W was not banded, and hence it was made up essentially of Alu I-sensitive euchromatin (fig. 4). The interphasic nuclei of females did not show any C-banding positive mass like that observed in other species of the family (Olmo et al., 1987). In *L. graeca* preparations treated with G-banding the W chromosome showed only a band at the telomeric level (fig. 5). Observations on *Ophisops elegans* were consistent with those reported by Bhatnagar and Yoniss (1976).

Heterogamety of the $Z_1Z_2W$ type was present in *Lacerta vivipara*. In the specimens coming from the Netherlands, the W chromosome was a metacentric macrochromosome (type B after Kupriyanova 1990; fig. 1). In preparations treated with C-banding, the W chromosome showed a centromeric band and two subtelomeric bands on both arms (fig. 4). In preparations treated with Alu I, the centromeric band and one of the two subtelomeric bands persisted, whereas the other subtelomeric band disappeared (figs. 1-4). In preparations treated with G-banding, two bands could be observed, one on the centromere and the other at the subtelomeric level, which might correspond to the Alu I-resistant bands (fig. 3).

Alu I treatment and G banding showed that $Z_1$ and $Z_2$ were two acrocentric chromosomes with different sizes. $Z_1$ was larger and had G-banding similar to the Z of other species, such as *P. sicula* (see fig. 3). Thus it can be considered as the original Z. It was longer than one of the two arms of W. A resemblance between one of the W arms and the proximal portion of $Z_1$ and between the other W-arm and the $Z_2$ was also observed in G-banding (fig. 3). These observations suggest that in *L. vivipara* W originated by centric fusion of an autosome and either sex homolog, which, however, would have undergone deletion of the distal portion before fusion.

In *L. vivipara* specimens coming from the USSR, W was an acrocentric or subtelocentric chromosome, and its size was the same as that of the biarmed W chromosome present in specimens from western Europe (type A after Kupriyanova, 1990; fig. 1). In the unarmed W chromosome, C-banding showed a C-negative centromere and two wide C-positive areas at the intercalary and telomeric levels (fig. 1).

In a second group of species it was not possible to find sex chromosomes by standard techniques but only by particular techniques, such as C-banding, treatment with the restriction enzyme Alu I, and G-banding. In *Algyroides moreoticus, A. nigropunctatus, L. agilis* and *Podarcis hispanica*, the W chromosome differed in being completely heterochromatic and C-banding was positive (fig. 2). In the preparations treated with Alu I, C-bands did not disappear on W, indicating that it was constituted by Alu-I-resistant heterochromatin (fig. 4). In *P. hispanica*, W appeared as large as Z, whereas in *L. agilis* and *Algyroides* it was smaller, being intermediate between a macrochromosome and a microchromosome, as in *L. viridis* (Olmo et al., 1987). Female interphasic nuclei in these species showed a C-banding-positive mass, persisting also after the treatment with Alu I. In *P. hispanica*, W showed a euchromatic intercalary area similar to that observed in other lacertids (Olmo et al., 1987). However, in this species, it was remarkably larger.

A peculiar situation was observed in *P. sicula*. In this species it was possible to find sex chromosome only by the G-banding technique, which allowed a more precise identification of the various pairs of homologs. In fact, W did not appear so morphologically different from Z either to be identified in preparations stained with traditional techniques or to be completely or widely heterochromatic and C-banding-positive.

G-banding studies showed heterogamety of the ZW type also in this species, where

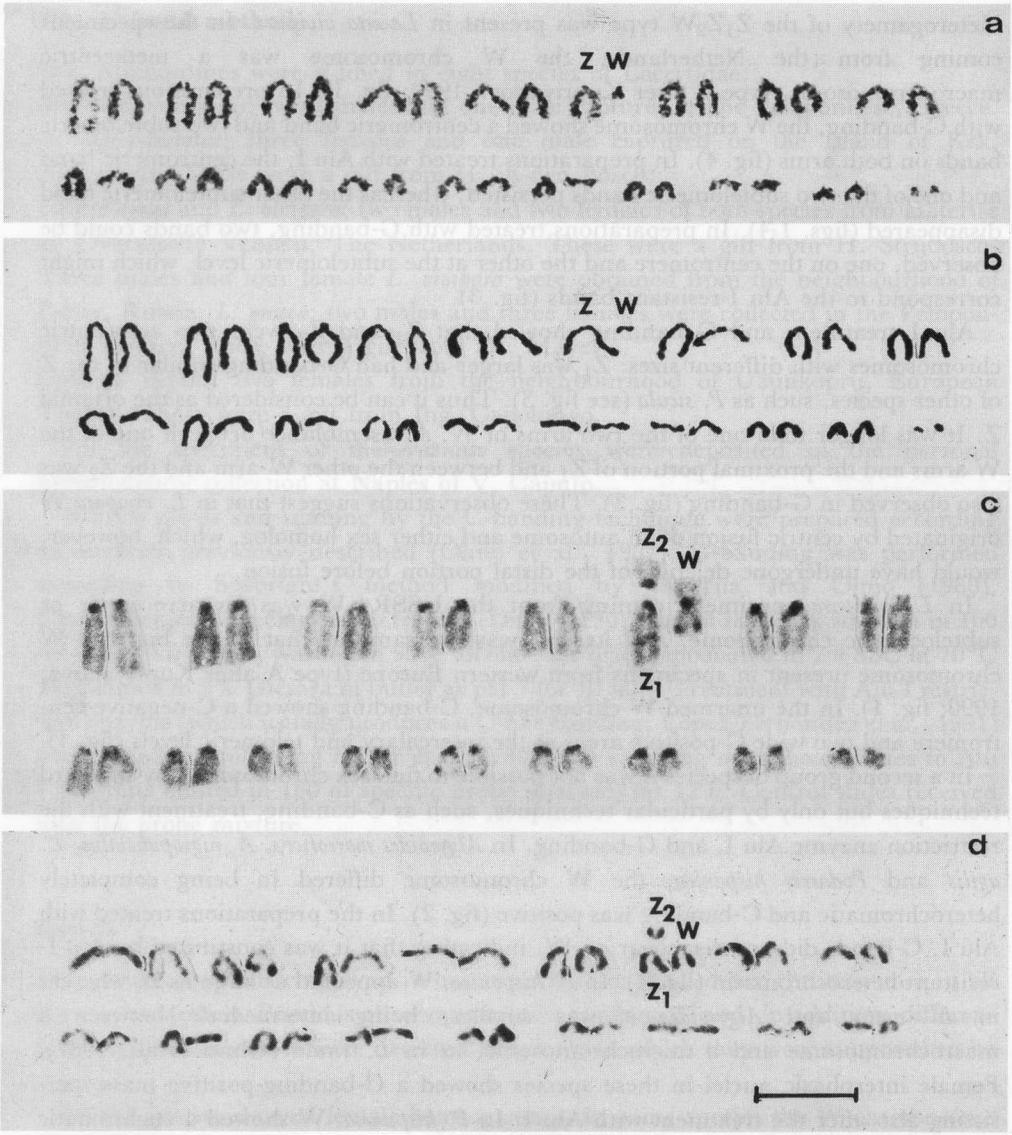


**Figure 1.** a) Karyotype of *Lacerta graeca*; b) Karyotype of *Ophisops elegans*; c) Alu I treated karyotype of *Lacerta vivipara* from Holland; d) C-banded karyotype of *L. vivipara* from U.S.S.R. Bar represents 10μm.

Z was a medium-large macrochromosome, whereas W was one of the smallest macrochromosomes showing two G-positive bands: an intercalary band and a telomeric band (fig. 3). In preparations treated with C-banding or Alu I, W did not appear banded, or, at the most, showed a small centromeric band, like other small chromosomes (fig. 4). In addition, no C-banding mass was observed in female interphasic nuclei.

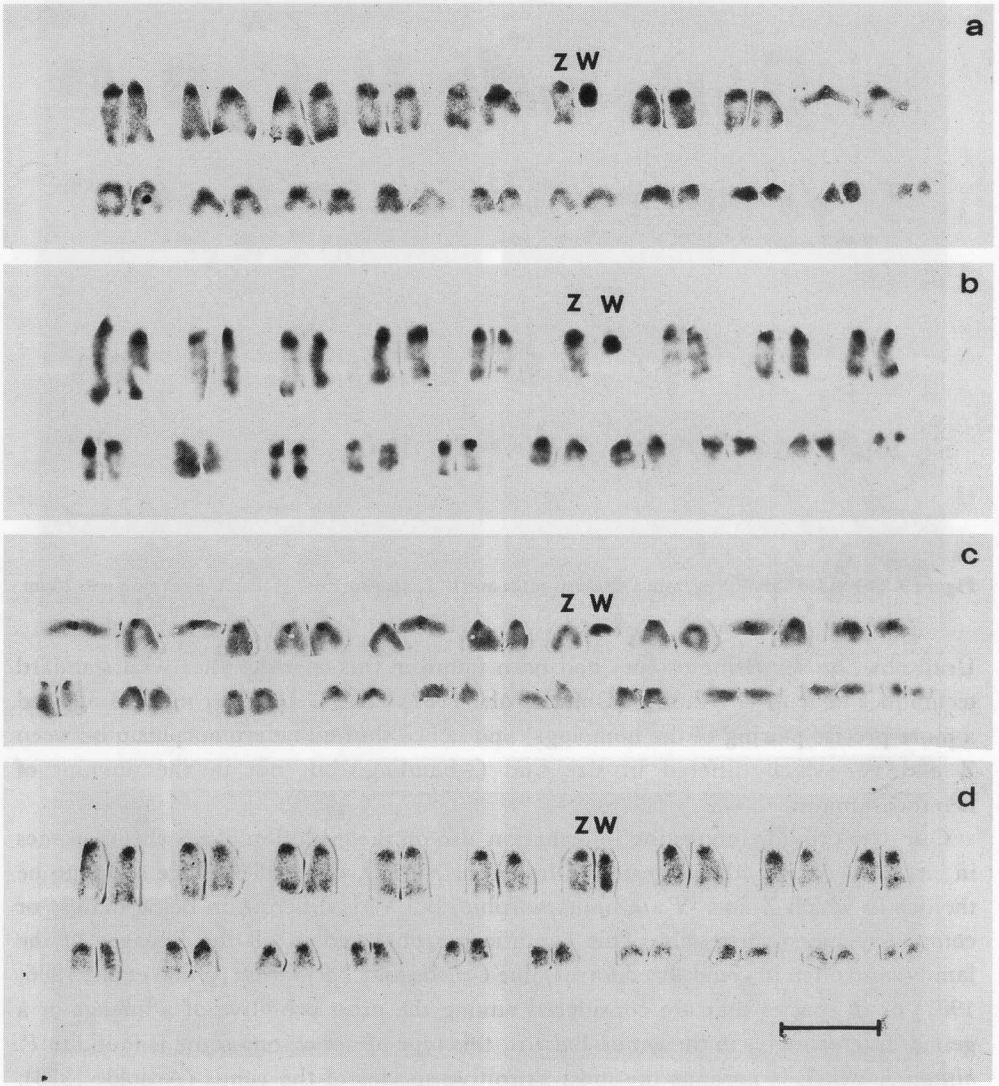


**Figure 2.** C-banded karyotypes from a) *Algyroides moreoticus*; b) *A. nigropunctatus*; c) *Lacerta agilis* and d) *Podarcis hispanica*. Bar represents 10μm.

## Discussion

Our results and data existing in the literature (Capula et al., 1989; Olmo et al., 1987; Odierna et al., 1990; Volobouev et al., 1990) suggest that sex chromosomes are likely to be present in all Lacertidae, though with different morphologies and at different degrees of differentiation; when they seem to be absent, this is probably due to the inadequacy of the techniques used. In this regard, *P. sicula* may serve as an example.

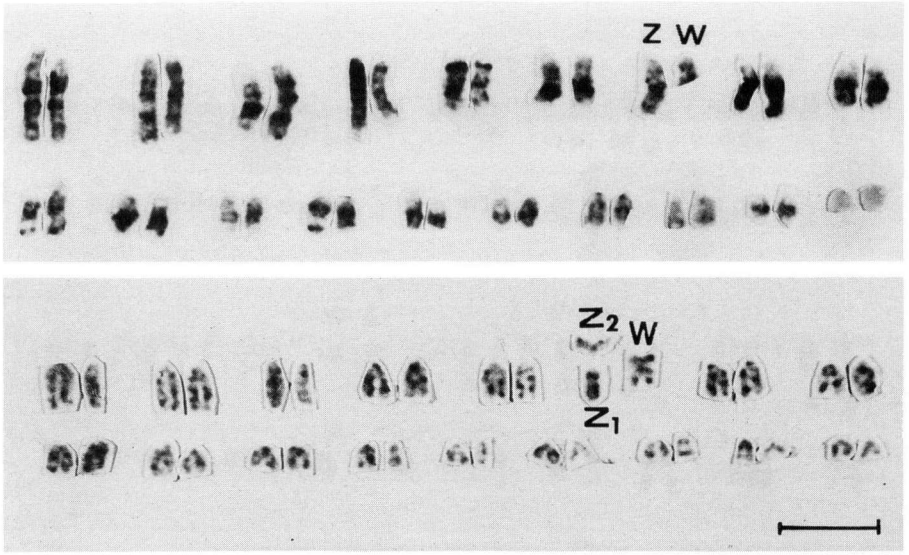


**Figure 3.** G-banded karyotypes from a) *Podarcis sicula* and b) *L. vivipara* from Holland. Bar represents 10μm.

Until now, no sex chromosomes had been found in this species either with standard techniques or with C-banding (Olmo et al., 1987). The G-banding method allowed a more precise pairing of the homologs, and hence showed heteromorphism between Z and W, which differed in size and G-banding, but not in the amount of heterochromatin.

Our data provide interesting information also on the evolution of sex chromosomes in lacertids. As already suggested (Olmo et al., 1987), the earliest stage seems to be the one in which Z and W are homomorphic, but W is different in being mainly or completely heterochromatic. This condition is observed in all the lineages of the family, and often in generalized forms, like *Gallotia* and *Takydromus* (Olmo et al., 1986, 1987) or in species that are considered among the most primitive of a lineage or a genus. Interestingly, in the genus *Podarcis*, this type of sex chromosome is found in *P. hispanica*, which is perhaps the most primitive species of the genus (Arnold, 1973); more advanced species, like *P. sicula*, have clear heteromorphic sex chromosomes.

The heterochromatic homomorphic W of some lacertids is the same as that found in several other groups of organisms, like snakes and birds (Olmo, 1986; Shields, 1983), where it is also considered as the initial stage of sex-chromosome differentiation (Jones, 1984). In addition, the presumed primitive condition of sex chromosomes in lacertids agrees well with Jones and Singh's hypothesis (Singh et al., 1980; Jones, 1984). They propose a model of sex chromosome differentiation where the starting point would be the isolation of sex-linked genes on sex-chromosomes through suppression of crossing-over as a consequence of accumulation of a sex-specific satellite DNA on either homolog, accompanied by wide heterochromatinization.

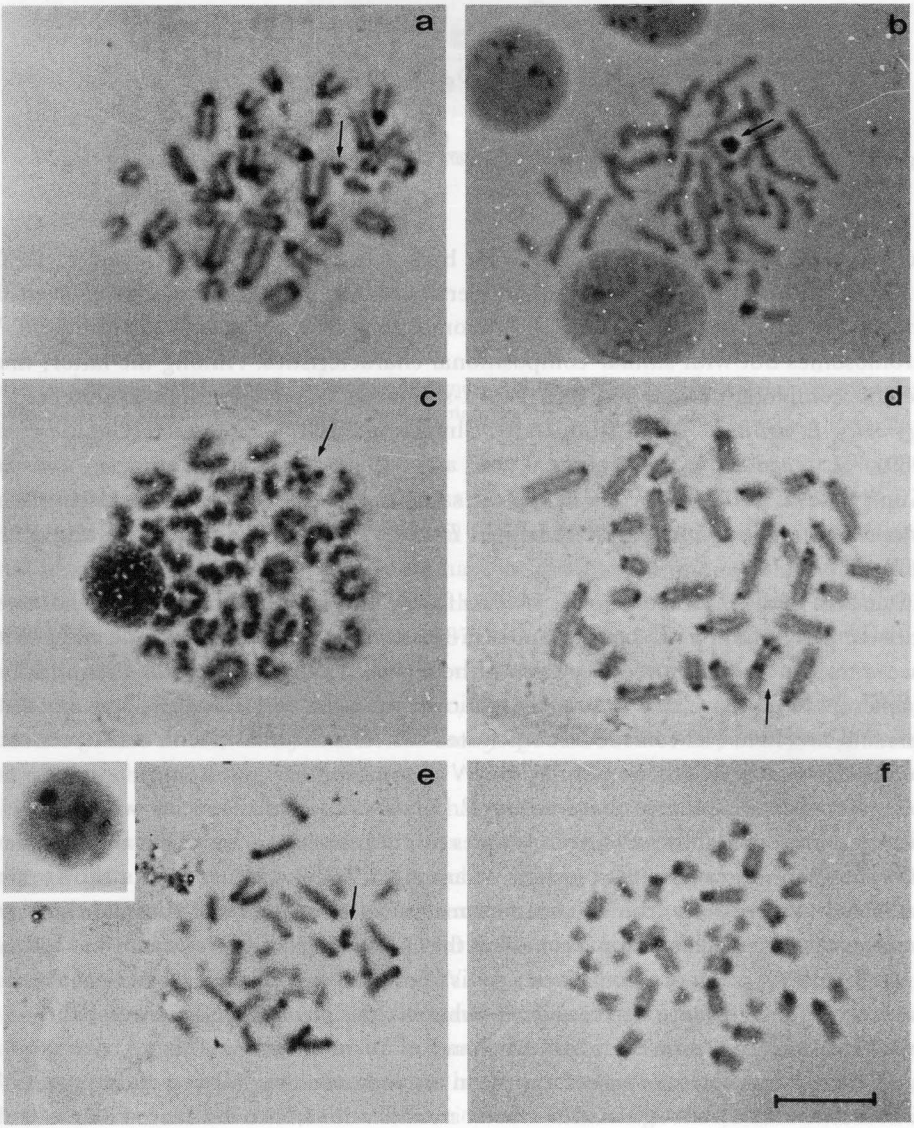


**Figure 4.** Alu I treated chromosomes from a) *L. graeca*; b) *L. agilis*; d) *L. vivipara* from Holland; e) *P. hispanica* (note the heterochromatic body in the interphase nucleus in the inset) and f) *P. sicula*. c) C-banded chromosomes of *L. vivipara* from Holland. The arrows indicate the W chromosomes. Bar is 10μm.

The primitive W of lacertids always shows two wide C-banded areas: a proximal and a distal, which are separated by a euchromatic area (Olmo et al., 1987) that, in *P. hispanica* is wider than in the other species. These C-banded areas are resistant to the enzyme Alu I and stain intensely with DAPI (Olmo et al., 1987), which may be

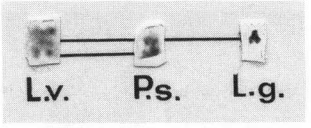


**Figure 5.** G-banded W-chromosomes from *L. vivipara* (L.v.), *P. sicula* (P.s.) and *L. graeca* (L.g.).

indicative of the presence of an A-T-rich highly repeated DNA (Schweizer, 1980).

A chromosome possessing these characteristics is found in many species of lacertids, whereas other species show nearly homomorphic or morphologically different W chromosomes but with similar compositional characteristics. Among the latter, there are the completely C-banded and DAPI-stainable W smaller than Z observed in *Algyroides, L. agilis, L. viridis* from Italy (Olmo et al., 1987), *L. horvathi* (Capula et al., 1989), *L. andreansky* (Volobouev et al., 1990) and *Archaeolacerta armeniaca* (Kupriyanova, 1989). A W of a nearly the same size of Z was observed in *Archaeolacerta rostombekovii* (Kupriyanova, 1989) and in *Eremias grammica* (Kupriyanova and Rudi, 1989).

One arm of the biarmed W of *L. vivipara* from Holland also shows similar characteristics. In fact, it has two C-banded and Alu-resistant areas, a proximal one and a distal one, with a euchromatic area separating them (figs. 1 and 4). The other arm displays a different situation: the heterochromatic distal area is C-banded, but not Alu-resistant, and hence it seems to be constituted of heterochromatin with a different composition from that of the homomorphic W chromosome.

Greater differences are observed in *P. sicula* and in the species where W is a microchromosome whose sex chromosomes might represent a more advanced stage of differentiation. However, a comparison among the G-banding results shows some similarities also between the W chromosomes of these species and those of the above mentioned species. This is apparent when the G-bandings of *L. vivipara* from Holland, *P. sicula* and *L. graeca* are compared. As is shown in fig. 5, the G bands of *P. sicula* may correspond to those of *L. vivipara*, whereas the proximal G-positive band of *P. sicula* may correspond to the telomeric band of W in *L. graeca.*

All these observations, therefore, seem to indicate that all lacertids possess W chromosomes that, though with various modifications, can be traced back to the homomorphic W.

Based on these observations, a model of sex-chromosome evolution in lacertids is proposed here (fig. 6). According to this model, sex-chromosome differentiation in lacertids may have started with the formation, on either homolog, of two heterochromatic areas, a proximal and a distal, which may have resulted in an inactivation effect. This would have lead to a W chromosome similar to that of *P. hispanica*, from which, by complete deletion of the distal heterochromatic area, a smaller but mainly euchromatic W would have originated, like that of *P. sicula*. A further deletion would have changed this chromosome into a microchromosome, like that found in

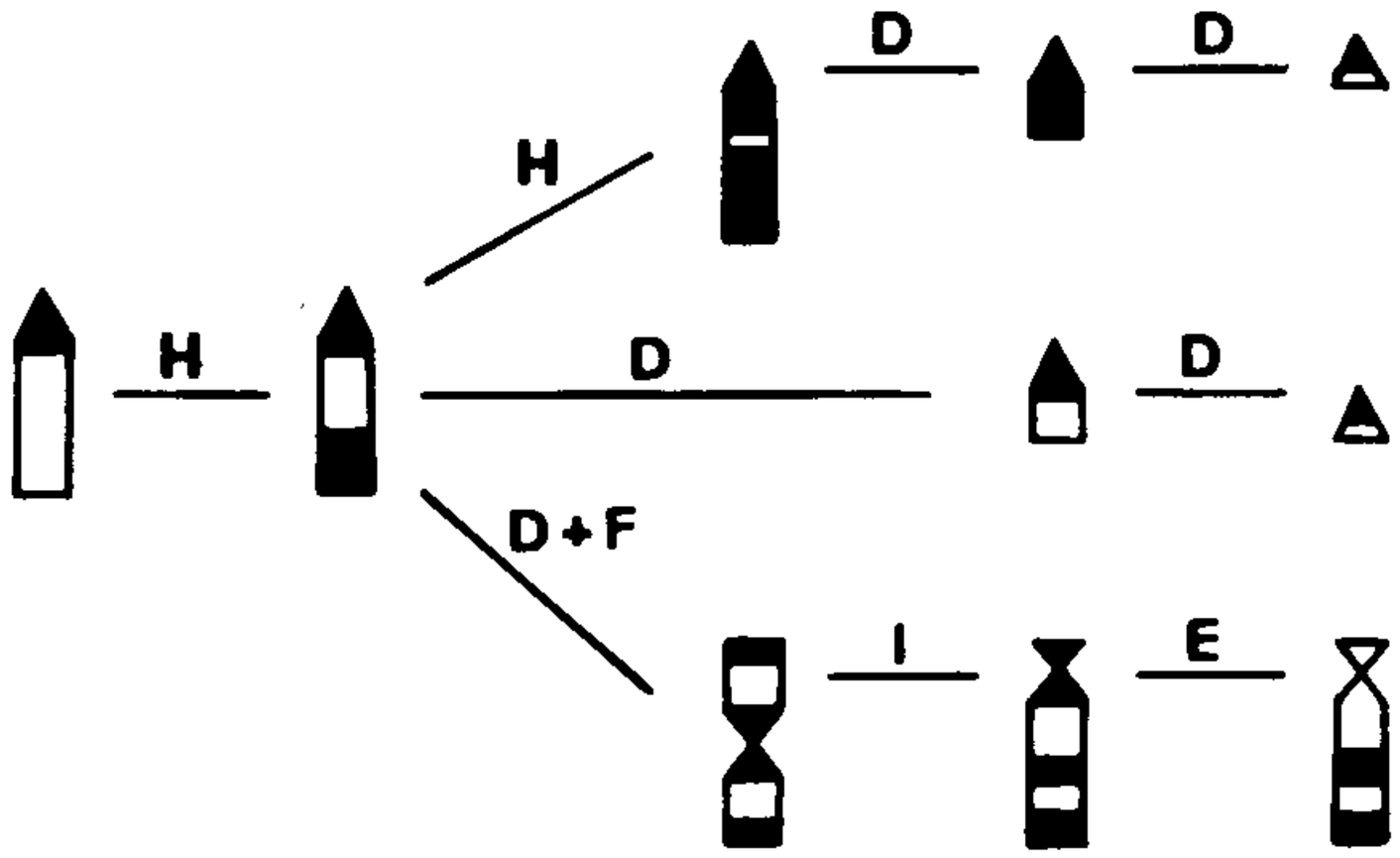


**Figure 6.** An hypothetic model of sex-chromosomes in lacertid lizards. H = heterochromatinization; D = Deletion; F = Fusion; I = Inversion; E = Euchromatinization.

some species of *Podareis* (De Smet, 1981) and in species related to it, like *L. graeca.*

In other lineages, the primitive W would have undergone a further more extensive distal heterochromatization which would have originated a homomorphic W with an interstitial euchromatic area smaller than that of *P. hispanica*, as observed in *Gallotia*, *Takydromus*, *L. lepida* etc. (Olmo et al., 1987). In this case too, a progressive deletion would have followed, which would have transformed W into a macrochromosome smaller than Z but mainly heterochromatic, similar to that seen in *Algyroides*, *L. agilis*, *L. viridis* (Olmo et al., 1987) and *L. andreanskyi* (Volobouev et al., 1990), and finally into a microchromosome.

In *L. vivipara* the evolution of W would have been more complex. The resemblance between the banding of one of the W arms and the homomorphic W of other species suggests that in this species also sex-chromosome differentiation started with heterochromatin accumulation on either sex homolog. This homolog also would have undergone deletion of its distal portion, and then would have joined with an autosome by centric fusion originating the biarmed W typical of *L. vivipara* from western Europe (type-B W after Kupriyanova, 1990). A further stage of this process would have been characterized by pericentric inversion accompanied by euchromatinization of the centromeric area (this latter phenomenon has been reported also for other species; King, 1990), which would have led to the formation of a uniarmed W like that observed in *L. vivipara* from Eastern Europe and Asia (type A after Kupriyanova, 1990).

An alternative hypothesis based on biogeographical considerations by one of us (Kupriyanova, 1990; Kupriyanova and Rudi, 1991) suggests that type A is the most primitive form. It would have originated by tandem fusion between a heterochromatic homomorphic W and an autosome. A subsequent pericentric inversion, accompanied by centromeric heterochromatization, would have led to form B.

Our results seem to give further support to the hypothesis that sex-chromosome differentiation in lacertids has taken place repeatedly and independently in different taxa (Kupriyanova, 1986; Olmo et al., 1987). In fact, W chromosomes at different stages

of differentiation and with different morphologies are found in specimens of various species.

In the most extensively studied species, the different types of sex chromosomes have different distribution areas. An example is provided by *L. vivipara*: specimens with type-B W chromosomes and those with type-A W chromosomes have clearly distinct distribution areas which are separated by the Carpathians (Kupryianova, 1990; Kupriyanova and Rudi, 1990). Likewise in *L. viridis*, specimens from France appear to have homomorphic sex chromosomes (Chevalier, 1969; Chevalier et al., 1979), whereas in Italian specimens the W chromosome is apparently smaller than the Z (Olmo et al., 1987). In addition, according to our preliminary observations, which are however to be confirmed, the W would be a microchromosome in specimens of *L. viridis* coming from Greece (Olmo et al., unpublished). These observations would suggest a polytypy related to sex chromosome differentiation which would have accompanied the progressive spreading of some species from their area of origin.

Cases of polytypy have been observed in sex chromosomes of various species of reptiles (Kasahara et al., 1983; Moritz, 1984, 1990). However, cases of sex-chromosome polymorphism are also known, i.e. of differences between speciemens of the same population (Moritz, 1990). Unfortunately, several intraspecific differences observed in the sex chromosomes of Lacertidae often concern specimens of unknown origin, and therefore do not provide further information on this aspect. Thus we cannot rule out that there are cases of polymorphism in the sex chromosomes of lacertids. Since the different types of W differ mainly in size, this would be due to unstableness of the heterochromatic W and its tendency to lose the heterochromatic areas which are not involved in sex determination.

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