FINE RESOLUTION OF THE KARYOGRAM OF *LACERTA SICULA CAMPESTRIS* (DE BETTA)

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The chromosomal constitution of reptiles, especially the presence or the absence of recognizable sex chromosomes, has been investigated for a long time by Japanese and Swiss students. After PAINTER (1921) and DALCQ (1920, 1921) who mistakenly reported a case of male heterogamety, NAKAMURA (1927, 1928, 1931) found isomorphic sex chromosomes that were nevertheless distinguishable because of their heteropicnosis in the premeiotic stages in several snakes and lizards. Female heterogamety of the ZO type was observed by OGUMA (1934, 1937) in *Lacerta vivipara* and *Amyda japonica* and by MAKINO and ASANA (1948) in two tortoises. On the other hand, MATTHEY (1931-1957), MARGOT (1946), MATTHEY and VAN BRINK (1956-1960) and VAN BRINK (1959) affirmed the absence of heteromorphic chromosomes in the whole class of reptiles.

Recently, numerous new contributions to the knowledge of reptile karyology, have furnished interesting data, frequently conflicting with previous reports. In fact, while KOBEL (1962, 1963) clearly demonstrates the presence of ZW chromosomes in the female of *Vipera berus* and *V. aspis,* and BEÇAK, BEÇAK and NAZARETH (1962) find them in *Bothrops jararaca* and in other *Serpentes* (BEÇAK, 1967). Male heterogamety is also found in some species of *Anolis* (GORMAN and ATKINS, 1966), *Sceloporus* (LOWE, WRIGHT and COLE, 1966; COLE, LOWE and WRIGHT, 1967) and in *Polychrus marmoratus* (GORMAN, ATKINS and HOLZINGER, 1967). Therefore karyological investigations on reptiles continue to be of great interest, and the resolution of the karyotype of a still unknown Lacertilian, is fully justified.

MATERIALS AND METHODS

We used the testicles, spleen and bone marrow of thirty-eight specimens (18 ♂ and 20 ♀) of *Lacerta sicula campestris* (de Betta) previously treated with a 1 : 20.000 Colcemid solution for about 12 hours.

After hypotonization in Na-cytrate solution, the finely fragmented testicles were fixed in methanol-acetic acid 3 : 1. The suspension was rubbed and then dried over a flame. Little fragments of spleen and bone marrow, previously hypotonized, were fixed in 50% acetic acid, and squashed by the usual technique. The preparations were coloured with Giemsa (Merck) in a 4% water solution.

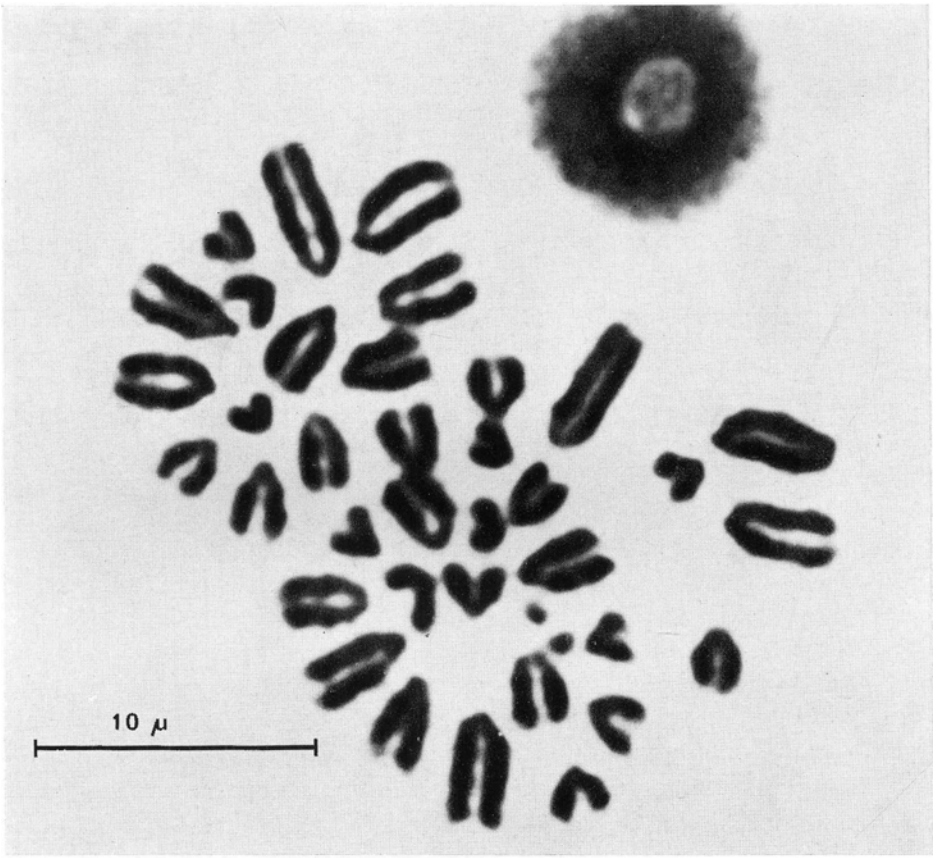

Fig. 1. — Metaphase nucleus of a testis cell.

RESULTS

The karyotype of *Lacerta sicula campestris* in both sexes shows 38 chromosomes; 36 are macrochromosomes and one pair is of microchromosomes (Fig. 1 and 4). All the chromosomes are telocentric and because of the perfect pairing of all 38 chromosomes in both sexes, it is not possible to establish which pair is that of the heterochromosomes. There is therefore no morphologically evident heterogamety (Fig. 2 and 5). The average lengths

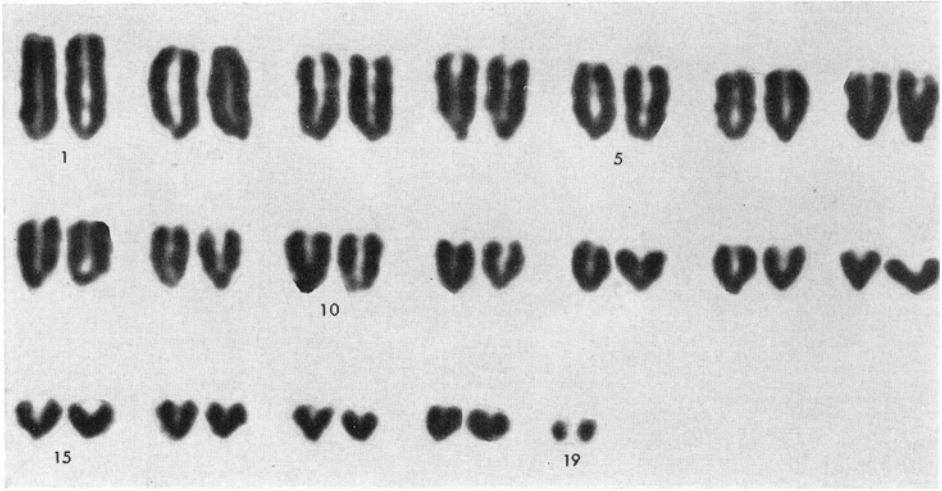

Fig. 2. — *Lacerta sicula campestris,* karyogram of the male.

of the chromosomes are slightly greater in the male: this is due to the different technique employed in the preparation of testicle and spleen. If we use the percentile ratio of the addition of the lengths of the chromosomes in their absolute value, the values are very similar in both sexes (see table).

TABLE I

No. chrom	lenght ♂ μ	ratio %	lenght ♀ μ	ratio %
1	5.35	9.07	4.37	9.71
2	5.07	8.60	4.01	8.91
3	4.69	7.95	3.82	8.48
4	4.24	7.19	3.34	7.42
5	4.02	6.81	3.18	7.06
6	3.78	6.41	3.03	6.73
7	3.63	6.15	2.81	6.24
8	3.52	5.97	2.61	5.80
9	3.26	5.53	2.49	5.53
10	2.97	5.03	2.32	5.15
11	2.64	4.47	2.03	4.51
12	2.45	4.15	1.87	4.15
13	2.34	3.96	1.78	3.95
14	2.24	3.79	1.62	3.60
15	2.12	3.59	1.48	3.28
16	2.01	3.40	1.35	3.00
17	1.88	3.18	1.27	2.82
18	1.81	3.07	1.16	2.57
19	0.93	1.57	0.66	1.46

It is practically impossible to group the pairs of homologous chromosomes on the basis of their length; in fact it diminishes gradually (see Fig. 3-6) from chromosome 1 to 18, and we can only observe a little gap between the last pair of macrochromosomes and the one pair of microchromosomes.

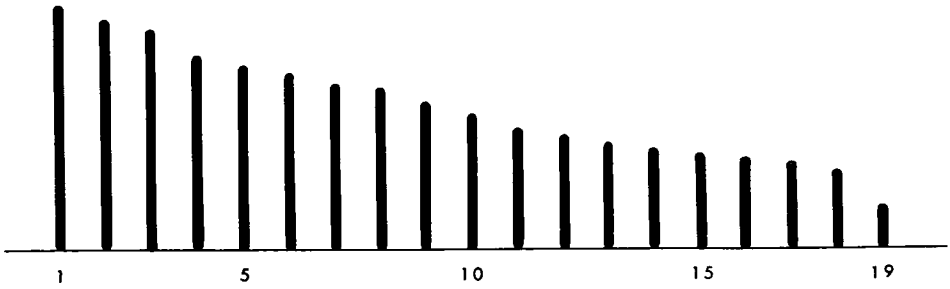

Fig. 3. — *Lacerta sicula campestris,* idiogram of the male.

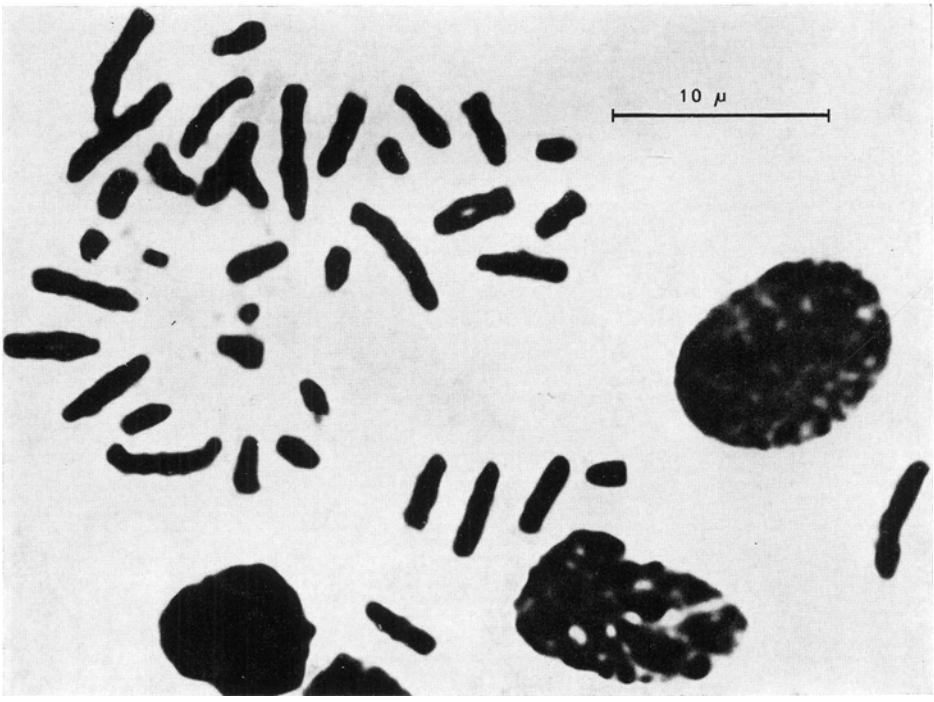

Fig. 4. — Metaphase of a spleen cell female.

In the male specimens treated with highly concentrated Colcemid, we also observed several anomalous mitoses with 76 chromosomes (tetraploidy), and clear images of « butterfly » chromosomes in meiotic anaphase problably due to a c-mitotic action.

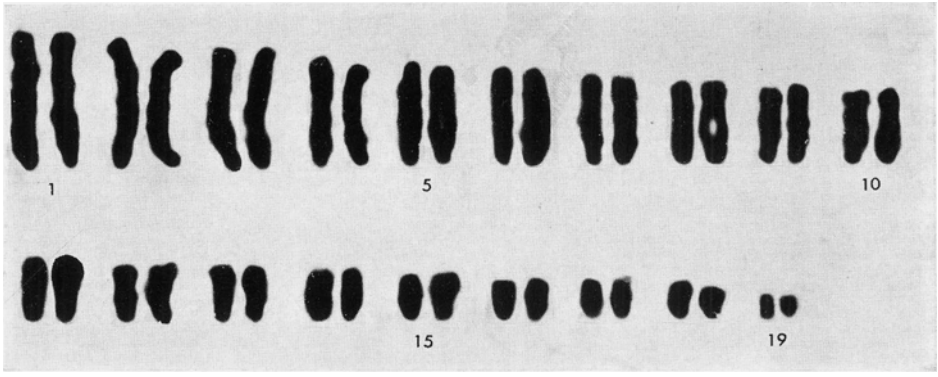

Fig. 5. — *Lacerta sicula campestris,* karyogram of the female.

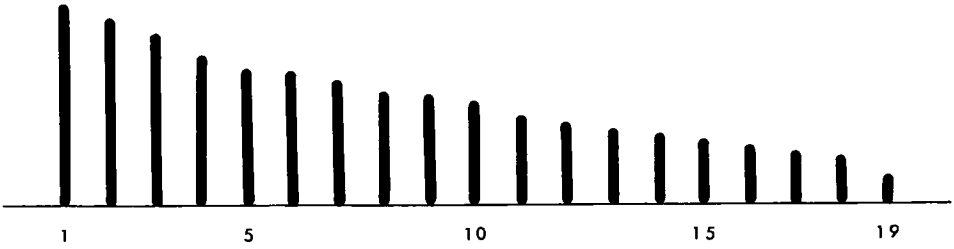

Fig. 6. — *Lacerta sicula campestris,* idiogram of the female.

DISCUSSION AND CONCLUSIONS

This investigation has established that the diploid number of *Lacerta sicula campestris* is $2n=38$.

Both the chromosomal constitution and the idiogram of this species are the same as those of *Lacerta muralis* (MATTHEY, 1931), *L. agilis, L. viridis, Psammodromus hispanicus, Tropidosaurus algirus, Takydromus* sp. (MATTHEY, 1945). *Lacerta ocellata* shows a karyogram that differs from that of the above-mentioned lizards in the number and morphology of the chromosomes (MATTHEY, l. c.): accepting Robertson's hypothesis of the centric fusion of two pairs of telocentric chromosomes, we can consider $2n=38$ to be the true chromosome number also for this species.

Only *L. vivipara* has $2n=36$ instead of the fundamental number $2n=38$, but MATTHEY (1951) does not seem to attach a great genetic importance to the absence of a pair of microchromosomes in this species. The presence in *L. sicula* of a chromosomal constitution that is identical to that of other species of the same genus, gives value to the hypothesis that, in reptiles, species of the same genus have the same chromosomal constitution (MATTHEY, 1943). The eventual exceptions to this general rule, may perhaps indicate the opportunity of a systematic revision of the group in which they have been observed: actually, quite recent karyological observations (GORMAN, 1965) tallied perfectly with the division of the genus *Anolis*, based on skeletal characters.

We have demonstrated the absence of heteromorphic chromosomes in *Lacerta sicula*, confirming the fact that morphologically recognizable sex chromosomes are absent in lizards. But heterogamety, both in the male and the female, has now been clearly demonstrated for other reptiles.

It is difficult, in the light of present knowledge, to interpret this wide variability in sex determination within the same class, while the other Amniota, birds and mammals, show a now stable chromosomal constitution as regards heterogamety. We may interpret the problem by recalling that all three groups originated from reptilian ancestors, but a great deal of work must still be done in the field of cytology before we can understand the steps in the evolution of sexual determination and in particular when the differentiation of the heterochromosomes in the higher classes of vertebrates, occurred. Many students are carrying out this research with promising results (BEÇAK, BEÇAK NAZARETH and OHNO 1964, ATKIN, MATTINSON, BEÇAK and OHNO 1965, a.o.).

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SUMMARY

The diploid number of *Lacerta sicula campestris* is $2n=38$. All the chromosomes are telocentric and morphologically recognizable heterochromosomes are absent.