Ascorbic acid synthesis in chick embryos

Age	Mesonephros	Metanephros	Livers	Yolk sac membrane
6-day	–	–	–	0.78 (2,194.0)
11-day	1.46 (85.5)	0.05 (3.0)	0.36 (114.1)	1.33 (11,180.0)
14-day	6.14 (518.7)	2.04 (353.3)	0.35 (309.0)	1.03 (15,489.0)
18-day	1.32 (57.4)	5.79 (2,788.1)	0.06 (141.2)	1.87 (25,197.0)

Ascorbic acid synthesis is measured in μmoles/g wet wt/h, and μg/day/embryo (in parenthesis). Values represent averages of four determinations.

phate-buffered solution containing sodium deoxycholate and incubated with/without the substrate, L-gulonolactone according to the method of Ayaz, Jenness and Birney[7]. AA concentration was determined by the method of Roe and Kuether[8]. L-Gulonolactone oxidase activity was measured by the net increase of AA in the tissue homogenates.

Tissues studied for activity of AA synthesis included the egg white, yolk, allantoic sac membrane, yolk sac membrane, brain, liver, mesonephros, and metanephros. The liver, mesonephros and metanephros were too small and fragile for the analyses until the embryo was 11 days old. It was necessary to combine tissues from several embryos to obtain sufficient samples for the study.

Our results are summarized in the table. In spite of the high concentrations of AA found in the embryonic brains, no AA synthesis was indicated in the brain tissues. No AA synthesis was detected in yolk, white or the allantoic sac membrane.

L-Gulonolactone oxidase activity was found in mesonephros of 11-, 14-, and 18-day chick embryos. But in the 18-day chick embryos, the enzyme activity of mesonephros had decreased, coinciding with its degeneration. While very low enzyme activity was found in the metanephros of 11-day chick embryos, it increased as the embryo matured.

Interestingly, L-gulonolactone oxidase activity was consistently found in the yolk sac membrane throughout the development of the chick embryos. The capacity of total daily AA synthesis in yolk sac membrane was much higher than those found in the mesonephros or metanephros of the chick embryo. The yolk sac has been regarded as the reservoir of nutrients. Our finding suggested that yolk sac membrane also actively synthesizes the AA required by the rapidly growing chick embryo.

Although no AA synthesis was reported in livers of chicken, low levels of L-gulonolactone oxidase activity was detected in the livers of the chick embryos during various stages of development. This result seemed to support Rinaldini's speculation[5] that some embryonic tissues might be capable of AA synthesis but loss the capacity during the course of differentiation. Different species of birds have been shown to synthesize AA in kidneys or in livers according to their phylogenetic trend[9]. It seemed probable that AA synthesis in chick during embryogenesis parallels the phylogenetic patterns.

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C-band variability in some Lacertidae (Sauria, Reptilia)

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Summary. The chromosome C-banding pattern has been studied in four lacertid species possessing the same karyotype. The results obtained show a remarkable interspecific variability both in the amount and distribution of C-banded heterochromatin. This leads us to the speculation that alleged conservativeness in their karyology is probably due to inadequate resolution by the conventional cytological techniques. Moreover, it has been hypothesized that these variations of the C-bands play an important role in the evolution of this saurian family.

Key words. C-band; heterochromatin; lacertid lizards; chromosomes.

In the investigations carried out with standard cytological techniques, the lizards from the family *Lacertidae* are commonly considered to be conservative from a karyological viewpoint[2-5]. However, it has been observed that in several animal groups, karyotypes which are apparently the same from the point of view of conventional morphology, have proved to be extremely different when examined by banding techniques[6-8].

In this regard we have studied the C-banding pattern in four lacertid species possessing similar karyotypes, with 36 uni-armed macrochromosomes and 2 microchromosomes. The aim of this research was to test whether the apparent homogeneity in karyology by conventional techniques corresponds to a homogeneity in the distribution of heterochromatin.

Material and methods. The C-banding pattern was investigated in five female specimens of *Lacerta dugesii* from the island of Madeira; two male specimens of *Lacerta trilineata* from the Balkans; one female and two male specimens of *Podarcis sicula sicula* from the surroudings of Naples and three female and two male specimens of *Takydromus sexlineatus* from Thailand.

The animals were stimulated with two doses of phytohemagglutinin (0.02 ml/g b.wt) then they were treated with colchicine (0.01 ml/g b.wt) and sacrificed after 6–19 h under anesthesia

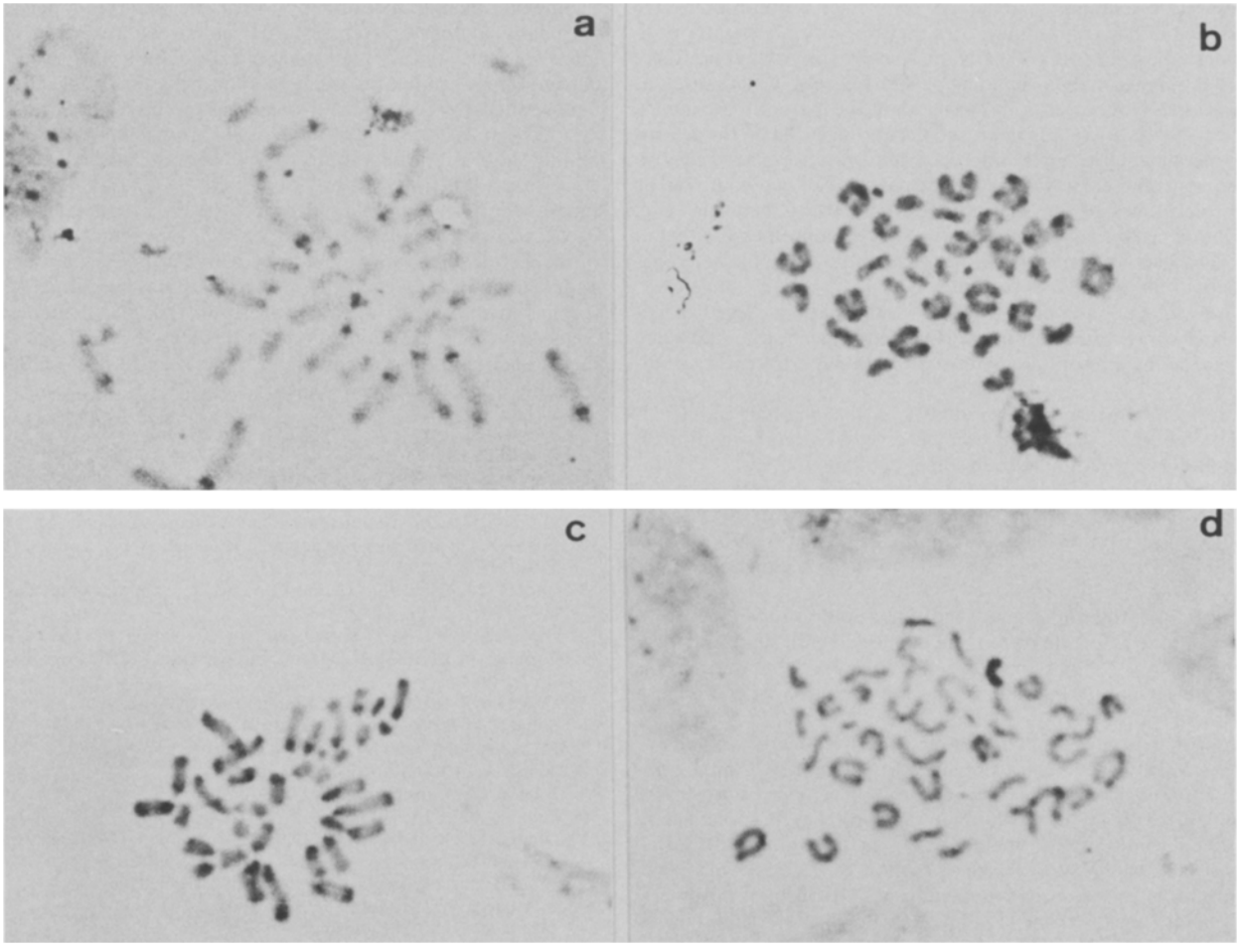

Figure 1. C-banded metaphase plates of: a) *Lacerta trilineata;* b) *Lacerta dugesii;* c) *Podarcis sicula sicula* and d) *Takydromus sexlineatus.* Note the completely heterochromatic W-chromosome of *T. sexlineatus.* ×1950.

with ethyl ether. The preparations were made with materials taken from the intestine and bone marrow, using the air-drying spreading technique described by Schmid[9].

The C-banding was performed by Sumner's[10] method, partially modified as reported by Olmo et al.[11]. The amount of heterochromatin was determined by measuring the area of the C-bands in relation to the total area of the chromosomes. The measurements were taken by means of an image analyzer Zeiss-Kontron, IBAS 1, on at least five plates per species.

Results and discussion. Figure 1 shows some C-banded metaphase plates of the four species investigated. The localization and the amount of the C-bands are schematized in the histogram of figure 2. The table reports the percentage area of the genome of each species occupied by C-banded heterochromatin.

The results obtained show a remarkable variability in the amount and distribution of the C-bands in the four species studied.

Lacerta trilineata has the lowest amount of heterochromatin. In fact, the C-bands account for only about 9% of the total genome area. They are observed on 9 out of the 11 pairs of larger chromosomes, and are mostly paracentromeric. However, some pale and doubtful telomeric bands are also found on the chromosomes of the first four pairs. Moreover, the microchromosomes show no C-bands. Conversely, a larger amount of heterochromatin is found in *Lacerta dugesii* and *Podarcis sicula,* both of which show about 25% of the total genomic area positively stained with the C-banding. In spite of a similar heterochromatin amount, each of these two species has its own pattern of C-banding. In *L. dugesii,* some chromosomes show centromeric bands and some paracentromeric ones, whereas clearly-evident telomeric bands can be observed in the chromosomes of the five larger pairs; unlike *L. trilineata,* the microchromosomes are completely and intensely C-banded. In *P. sicula* all banded chromosomes show centromeric bands and most of them pos-

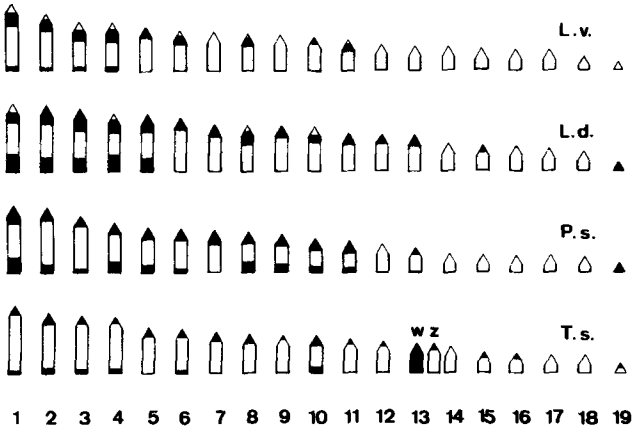

Figure 2. Idiograms of *L. trilineata* (L. v.); *L. dugesii* (L. d.); *P. sicula* (P. s.) and *T. sexlineatus* (T. s.), showing the localization of the C-bands (black). W and Z denote the sex chromosomes of *T. sexlineatus.*

sess clear telomeric bands too. The microchromosomes are banded although in some plates they appear only slightly positive to the C-banding. Finally, in *Takydromus sexlineatus*, there is a difference in the C-banding pattern between the two sexes, as was already reported[11]. In fact, both in the male and the female the C-bands are rather small and may be localized on the centromeres and telomeres; the microchromosomes are labeled only on the centromere. However, in the female, one of two homologous chromosomes of the 13th pair is completely C-band positive. This led us to suggest that they are sex chromosomes and that in this species a primitive and little differentiated female heterogamety of the type ZW is present[11].

The results of our study provide evidence that the alleged karyological conservativeness in lacertid lizards may be essentially due to inadequate resolution by the conventional cytological techniques.

Inter- and intraspecific variability in the amount and localization of the C-bands has been observed in several organisms[12], and it has been found also in some reptilian groups[7,8,13-15]. Generally, this variability is related to variations in highly repeated DNA fractions[12]; this may be true also for lizards. In fact the percentage of the heterochromatin in *Podarcis sicula sicula* (25%) corresponds approximately to the percentage of palindromic and highly repetitive DNA (22%)[16].

Several investigators suggest that inter- and intraspecific variations of the heterochromatin are connected with speciation phenomena[12,17,18]. It is therefore possible that these variations played an important role during the evolution of the present lacertid species.

Cobror[5] has suggested that some of the telomeric C-bands of *Lacertidae* result from a translocation of heterochromatic microchromosomes to the macrochromosomes. This translocation took place during the evolution of ancestral karyotypes richer in microchromosomes to the present lacertid karyotypes[2,5]. However, it is also possible that the quantitative variations of heterochromatin in the species studied, especially in the centromeric C-bands, result from amplification of pre-existing heterochromatic blocks. This phenomenon has been observed in several rodents[17,19] and in snakes[7]. It would seem to play an important role in transforming, through the addition of heterochromatin at the centromeric level, uni-armed chromosomes into bi-armed ones[17] and microchromosomes into macrochromosomes[7]. In this regard, it is noteworthy that our preliminary results show that in the two lacertid species *Gallotia galloti* and *Lacerta viridis*, some of the largest chromosomes possess a minute short arm, which seems to be C-banding positive (Olmo et al., unpublished).

Finally, the heterochromatinization of the W-chromosome seen in *Takydromus sexlineatus* might have an important function (unpublished observations made by us seem to show an analogous situation in *Gallotia galloti*). In fact, it has been suggested that, as in snakes[20,21], in this species also this phenomenon is connected with the accumulation of a specific sex-linked satellite DNA, and that it might represent a primary event in the differentiation of the sex-chromosomes[11].

Heterochromatin amount of the four species studied, expressed as a percentage of the total chromosomal area, positive to the C-banding. A statistical analysis, based on the Snedecor's F test, shows that the heterochromatin content of *L. trilineata* differs significantly from those of the other species.

Species	Heterocr. amount (%)	SE
Lacerta dugesii	25.99	± 2.11
Lacerta trilineata	9.09	± 1.08
Podarcis sicula	25.53	± 3.17
Takydromus sexlineatus	22.33	± 4.82

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Acetaldehyde oxidation in *Drosophila* null-mutants for alcohol dehydrogenase[1]

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Summary. Aldehyde dehydrogenase (ALDH) activity is demonstrated in four strains of *D. melanogaster* lacking active alcohol dehydrogenase (ADH-null mutants). In the four strains, ALDH activities are similar to those found in a wild strain. It is concluded that ADH-null flies are able to detoxify acetaldehyde. This finding is discussed in relation with the dual function of ADH proposed recently.

Key words. Acetaldehyde oxydation; alcohol dehydrogenase; aldehyde dehydrogenase; *Drosophila melanogaster; Drosophila simulans;* ethanol catabolism; null-mutants for alcohol dehydrogenase.