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Further data on the occurrence and evolution of satellite DNA families in the lacertid genome

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This paper reports the isolation and characterization of two *Hind*III repetitive DNA families from the genome of two lacertid lizards, *Podarcis sicula* and *Lacerta saxicola*. These satellites did not appear to be related to each other. The consensus sequences of their monomeric units did not show any similarity, though both DNAs were A-T rich. Moreover, each of them was found only in closely related species. The monomeric unit of the *Hind*III DNA family isolated from *P. sicula* (pLHS) showed a close resemblance to pLCS, a centromeric satellite DNA previously isolated from the same species; it was, however, mainly localized at pericentromeric, interstitial and telomeric levels. The results also provide interesting information on the systematics of the lacertids studied.

Key words: evolution, reptiles, satellite DNA

Introduction

Highly repeated DNA families localized on heterochromatic areas of the chromosomes (e.g. the centromere), used as probes, have proved an interesting and useful tool for determining the phylogenetic distances between related species (Macgregor 1990). Generally these DNAs tend to be preserved with time, and their sequences evolve proportionally to the divergence time between species (Miklos 1985). The study of these DNA fractions appears promising for elucidating lacertid phylogeny (Olmo *et al.* 1990). In fact, a satellite DNA tandemly arrayed on the centromere of nearly all the chromosomes of *Podarcis sicula* has provided interesting information on the phylogenetic relationships between some genera of the family, in particular on the position of the genus *Podarcis* (Capriglione *et al.* 1989, 1991).

In this study we further characterized highly repeated DNA families of lacertids. In particular, we investigated some satellite DNAs that are revealed in many species by the *Hind*III restriction enzyme, and are believed to be widespread in these saurians (Capriglione *et al.* 1990, Cardone *et al.* 1990).

Materials and methods

Animals and preparation of metaphase chromosomes

Individuals of *Podarcis sicula, P. muralis, P. taurica, P. tiliguerta* and *Lacerta bedriagae* were collected by Dr V. Caputo. Specimens of *L. dugesii, L. lepida* and *L. viridis* were purchased from an animal dealer. *L. vivipara* animals were a kind gift of Dr Strijbosch (Holland). *L. saxicola* specimens were kindly provided by Dr L. A. Kupriyanova (Russia).

Mitotic chromosome plates were prepared for *in situ* hybridization according to Olmo *et al.* (1986).

DNA extraction

Livers and gonads of all lizards were homogenized in cold saline solution (150 mM NaCl, 100 mM EDTA, pH 8.0), incubated overnight with sodium dodecyl sulphate 1% and protease K 0.1 mg/ml at 37°C. The proteins were removed by phenol–chloroform extraction.

Clone preparation

Monomeric units obtained by digesting *P. sicula* and *L. saxicola* genomic DNAs with *Hind*III, were ex-

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tracted from low melting agarose gels. The fragments were ligated to the *Hind*III cloning site of pUC 18 plasmid.

Southern analysis

DNA samples (10 µg) were digested with 2 U/µg of *Hind*III for 5 h and run on a 1.5% agarose gel. The gels were capillary blotted onto nylon membrane (Hybond-N, Amersham) according to Southern (1975). Hybridization of pLHS was carried out by a non-radioactive method. A 0.1 µg sample of the clone was labelled by random primer extension using digoxigenin-dUTP (Boehringer-Mannheim Kit). Southern hybridization and immunological detection were performed following the supplier's protocol.

^{32}P-labelling was used for Southern hybridization of the pSHS clone. Southern blots were washed under the same conditions as for pLHS.

In situ hybridization

The hybridization mixture contained 50% formamide, 2 × saline sodium citrate (SSC), 10 ng/µl digoxigenin-labelled probe and 0.1 mg/ml *E. coli* DNA. After denaturation in 70% formamide, 2 × SSC for 2 min at 70°C, the slides were incubated with the hybridization mix at 37°C overnight. Washings were carried out in 2 × SSC at room temperature and 37°C. Cytochemical detection was performed as suggested by the supplier (Boehringer-Mannheim).

The colour reaction was developed for 2 h, and the chromosomes were counterstained with orcein for 15 min.

DNA sequencing

The nucleotide sequence of the inserts contained in pLHS and pSHS was determined according to the dideoxy chain termination method (Sanger *et al.* 1977). Radiolabelling of the extended fragments was accomplished using [^{32}P]dATP (Amersham, 400 Ci/mmol) in the reaction mixture.

Results and discussion

Treatment with *Hind*III revealed a ladder of bands in almost all of the species investigated. We have verified in various experiments, using different enzyme concentrations and times of treatment, that the concentration of the enzyme and the treatment time reported in the materials and methods were enough to guarantee the complete digestion of the lizard genomic DNA. The ladder of bands is therefore indicative of the occurrence, in these species, of one or more highly repeated monomers of 150–180 bp (Figure 1A).

In order to verify that the sequence was always the same and to perform a closer analysis, we isolated the monomeric band from two species ascribed to two different genera, *Lacerta saxicola* and *Podarcis sicula*. DNA clones were obtained, referred to as pSHS (*saxicola Hind*III satellite) and pLHS (lizard *Hind*III satellite), respectively. Such clones were used as probes for hybridization by Southern blotting, the nucleotide sequence was determined, and pLHS was also used for *in situ* hybridization on chromosomes of *P. sicula* and the congeneric species *P. tiliguerta*.

As can be seen in Figure 2, pSHS hybridized only to the homologous DNA. The clone pLHS hybridized to the DNA of all the species of the genus *Podarcis*, but it failed to hybridize to the DNA of species ascribed to other genera (Figure 1B). Hybridization intensity was almost identical in *P. sicula* and *P. muralis*, whereas, in *P. taurica* and *P. tiliguerta*, low molecular weight bands were absent or, if present, showed weak hybridization signals.

In situ hybridization with digoxigenin-labelled pLHS showed that, in *P. sicula*, this DNA was centromerically or pericentromerically localized on

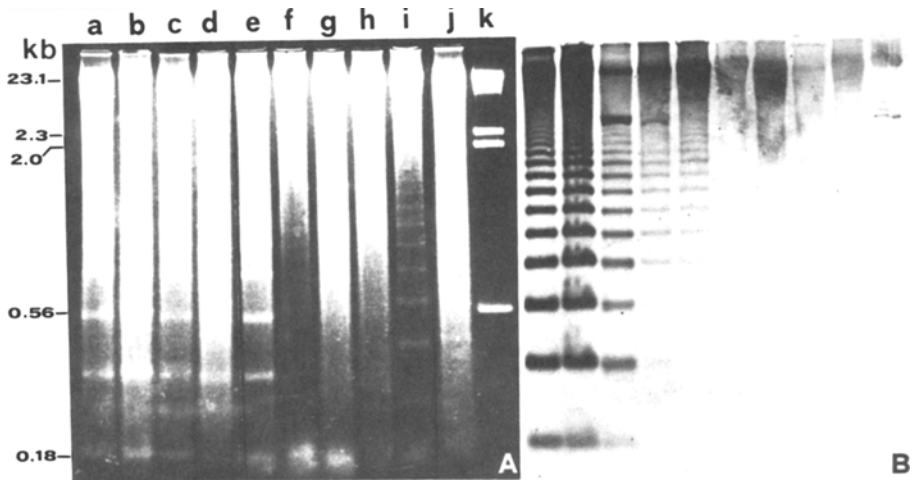

Figure 1. A Genomic DNAs of different lacertid species digested with *Hind*III: **a** *P. sicula* (Regi Lagni, Italy); **b** *P. sicula* (Punta Licosa, Italy); **c** *P. muralis*; **d** *P. taurica*; **e** *P. tiliguerta*; **f** *L. bedriagae*; **g** *L. dugesii*; **h** *L. lepida*; **i** *L. saxicola*; **j** *L. viridis*. **B** Hybridization of the same gel probing with digoxigenin-labelled pLHS.

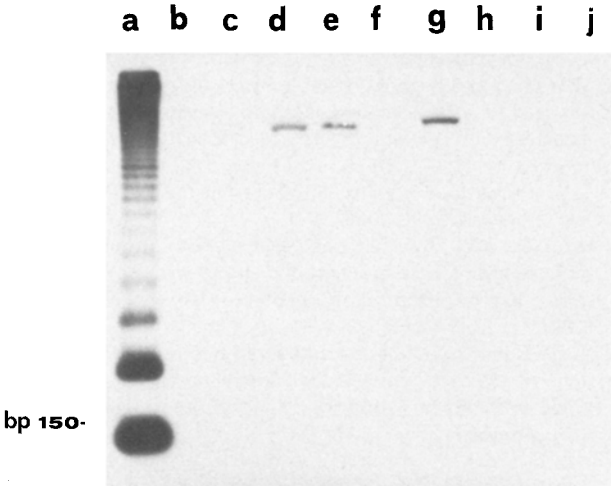

Figure 2. Southern blot of *Hind*III-digested DNAs probed with [^{32}P]pSHS: **a** *L. saxicola*; **b** *L. bedriagae*; **c** *L. dugesii*; **d** *L. lepida*; **e** *L. viridis*; **f** *L. vivipara*; **g** *P. sicula*; **h** *P. muralis*; **i** *P. taurica*; **j** *P. tiliguerta*.

almost all chromosomes, and in some of the largest it was also found at an interstitial and/or telomeric position (Figure 3A). In *P. tiliguerta*, it was almost exclusively pericentromerically localized (Figure 3B).

An analysis of the consensus sequences of the two satellite DNAs obtained from the various clones containing the basic repeats showed that both DNAs were rich in A-T (about 60%), but their sequences were very different. A comparison between pLHS and another satellite, pLCS, previously isolated from *P. sicula* (Capriglione *et al.* 1991) showed a close sequence identity (about 80%) between their sequences. This may suggest that these two satellite DNAs are variants of the same original sequence, but show different spread and chromosomal location.

The occurrence of two or more related sequences in the same species has been observed elsewhere (Baldini *et al.* 1992, Miller *et al.* 1993), and might be the consequence of a divergence event followed by the reamplification of the diverging sequences (Miklos 1985, Bostock 1986).

Our results showed the occurrence of a group of satellite DNAs in lacertids, all of which could be isolated with the *Hind*III enzyme, but which seemed to possess sequences very different from one another. Moreover, each is present only in closely related species. This was demonstrated by Southern blotting experiments and by the difference observed between the sequences of pLHS and pSHS (see Figures 1B, 2). These observations suggest that these *Hind*III satellite DNAs have different origins, or that they have a common origin but diverged very rapidly after separation of the different species.

Satellite DNAs having a common origin but differentiating early with species divergence, have already been observed (Miklos 1985, Lohe & Roberts 1988). Dover (1982) suggested that this might be the result of 'molecular drive', which would be dominant in the evolution of highly repeated DNA.

The analysis of the satellite DNAs, pLHS and pSHS, provides interesting information on the systematics of lacertids. The occurrence of pSHS only in *L. saxicola* indicates that this species is a taxon distinct from other species ascribed to the same *Archaeolacerta* group, such as *L. bedriagae* and *L. dugesii* (see Arnold 1989). Likewise the presence of pLHS only in the species of the genus *Podarcis* confirms that this genus is a clearly distinct natural group. The distribution of pLHS in the various *Podarcis* species studied is also interesting. Klemmer (1957) divided this genus into three groups: *muralis* including also *P. tiliguerta*, *bocagei* and *sicula* which would also include *P. taurica*. In agreement with Klemmer *et al.* (1977) and Guillaume & Lanza (1982), on the basis of immunological and electrophoretical studies, considered *P. muralis* and *P. tiliguerta* to be closely related to each other and distant from *P. sicula*. In contrast, Lutz & Mayer (1985), studying albumin evolution by microcomplement fixation, considered *P. sicula* and *P. muralis* to be closely related, and *P. taurica* and *P. tiliguerta* more distant.

As has been observed, pLHS shows almost the same hybridization in *P. sicula* and *P. muralis*, but

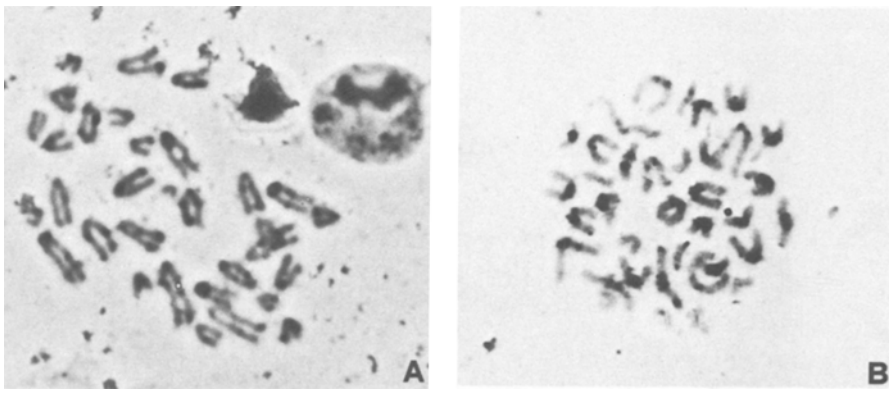

Figure 3. *In situ* hybridization of mitotic chromosomes of **A** *P. sicula* and **B** *P. tiliguerta* with digoxigenin-labelled pLHS.

lower hybridization in *P. taurica* and *P. tiliguerta*, where, moreover, lower molecular weight bands cannot be observed. This is clear evidence of divergent DNA sequences.

Our results are in contrast with the conclusions drawn by Klemmer (1957) and Lanza and co-workers (1977). They are instead, in good agreement with the observations of Lutz & Mayer (1985), suggesting the close phylogenetic affinity between *P. sicula* and *P. muralis*.

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