Comp. Biochem. Physiol. Vol. 100B, No. 3, pp. 641–645, 1991
Printed in Great Britain

0305-0491/91 $3.00 + 0.00

EVOLUTION OF A CENTROMERIC SATELLITE DNA AND PHYLOGENY OF LACERTID LIZARDS

TERESA CAPRIGLIONE,* ANNA CARDONE, GAETANO ODIERNA and ETTORE OLMO
Dipartimento di Biologia Evolutiva e Comparata, Universitá di Napoli, via Mezzocannone 8, 80134 Napoli, Italy (Tel: 081 552 5333); (Fax: 081 552 7807)

(*Received* 25 *March* 1991)

Abstract—1. The composition and phyletic distribution of a highly repetitive satellite DNA, isolated from *Podarcis sicula*, was studied.

2. This DNA was rich in adenine and thymine and displayed frequent adenine stretches. It was always located on the centromeric heterochromatin even in quite taxonomically distant species.

3. Southern blot hybridization of the Taq I satellite on various species of lacertid families showed a close affinity among *Podarcis*, *Algyroides* and *Lacerta dugesii*.

4. All the other taxa investigated did not seem to possess this repeated sequence.

INTRODUCTION

Our knowledge on the characteristics and functions of clustered satellite DNAs has been gradually improving, and this has aroused a greater interest in research on these DNAs.

Satellite DNAs located on heterochromatic areas (mainly on the centromere) are of particular interest; evidence indicates that they may play an important role in the process of heterochromatin condensation (Radic *et al.*, 1987).

In addition, satellite DNAs may also be used as a probe to estimate phyletic distances between related species, since the sequences of these DNAs have been usually seen to evolve proportionally to the time of divergence between species (Miklos, 1985; Lima de Faria *et al.*, 1984; Peacock *et al.*, 1977).

In the genome of the lizard *P. sicula*, we isolated a Taq I satellite DNA family, which is localized on the centromere of almost all the chromosomes.

This paper aims to develop a deeper insight into the study of this DNA in order to detect whether it can be related to the centromere functions or to the process of heterochromatin condensation, as well as to determine its distribution in the various species of the lacertid family and its evolution within this reptilian group.

MATERIALS AND METHODS

Animals and preparation of metaphase chromosomes

Individuals of *L. viridis*, *L. lepida* and *L. dugesii* were purchased from an animal dealer; *P. sicula*, *P. muralis*, *P. tiliguerta*, *P. taurica* and *L. bedriagae* were collected by Dr V. Caputo. Specimens of *A. moreoticus* and *A. fitzingeri* were kindly provided by Dr H. in den Bosch. Mitotic plates for *in situ* hybridization were prepared according to the technique of Olmo *et al.* (1986).

DNA extraction, Southern transfer and hybridization

DNA was isolated from livers and gonads of lizards according to Jeffreys and Flavell (1987). Ten μg of DNA of each individual was digested with Taq I restriction enzyme (BRL) according to the manufacturer's recommendations; they were then separated on a 1.2% agarose gel and transferred to a nylon filter according to the method of Southern (1975). Hybridization was carried out as described by Capriglione *et al.* (1989).

In situ *hybridization*

Hybridization of ^{3}H-labelled pLCS to mitotic chromosomes of *L. dugesii* was performed as described by Gall *et al.* (1971). RNase pretreated slides were denatured by boiling. Thirty microliters of hybridization mixture containing 50% formamide, 2 × SSC and 300,000 cpm of ^{3}H-labelled probe were used for each slide. Slides were then dipped in Ilford K2 emulsion and exposed for 30–40 days at 4°C.

DNA sequencing

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

RESULTS

Table 1 reports the consensus sequence of the monomeric unit of the pLCS satellite evaluated by the analysis of clones from different specimens and different populations of *P. sicula*.

pLCS DNA is 190 bp long, and, as shown in Table 2, is particularly rich in adenine–thymine (57%), and mainly in adenine, which accounts for 33% of the whole sequence. Repeat subunits were not present in this sequence. However, quite long adenine stretches were observed, which repeated quite frequently, even though not at regular intervals, and were always preceded by a guanine or cytosine. There were also thymine stretches, but these were less frequent than the adenine ones.

*Author to whom correspondence should be addressed.

Table 1. Consensus sequence and composition of four clones of the Taq I satellite

	10 20 30 40 50
pLCS-Cons 5′	CAACTTAACCGGCAGAAAAAGATTTTTTTGAAAAAATCCCTAAGG CCC
1	. . . . C.AT . . .
2	.T. . . . C. . .T. .G. . . . . C. . . . . .G.
3	G. T. T. . . C. CCT. .C .G.
4	. . . .
	60 70 80 90 100
pLCS-Cons	CCGGGGTCACAATTTCAGCAAAAA TCTGAC TTTCTCCA TTT CACT
1	. . . . TT. . . . . AA. . . A.
2	G . . . . .T. . . . . G.CG. . . . . .C. . . G. .
3	GT . . . . .G. . . . G. .A. G. . . . .
4	. . . . .TA. . . . . A. . . C. . . . . .GG. . . . .
	110 120 130 140 150
pLCS-Cons	CAAAAATGGTGCCAAACGCTTGCAAACTGCTT ACCCCCTGAAAGGTTT
1	A. . . . . . . .A . . . . .AG . . . .
2	. . . . .G. . . . . T. . .A. . . . . .C . . A . . . .
3	. . . . .TG. . . . . GA. . . . .
4	. . . . .G.A. . . . . G. . . . . . .G. . . . . .
	160 170 180 190 200
pLCS-Cons	GGCGGTCGAAACAGAAGGCTTTT ATCATTACAGGACAAGAAATCAGCCC
1	. . . . A . . . . .G.G
2	. . . . . .G. . . . .G . . . . G. . . . . C. . . . .
3	TT. . . .G . . . . T. . G. . . T . . . . A .T. G. . .T. . . . .
4	. . . . .C. . . . . 3′

By comparing clones from different populations we determined the consensus sequence and the number of unchanged and changed bases.

The pLCS sequence was seen to be quite constant, particularly in the A–T richer regions and in the long adenine stretches (Tables 1 and 2). In fact, Table 2 shows that the most variable bases are cytosine and guanine, whereas more than 60% of adenine and thymine, and in particular all the adenine stretches, are preserved.

Digestion of genomic DNA with Taq I in several species ascribed to the genera *Podarcis*, *Algyroides*, *Lacerta* part I and *Lacerta* part II, showed a regular ladder of low mol. wt bands in all the species of *Podarcis* and *Algyroides*, in *L. dugesii* and, though less clearly, in *L. viridis* (Figs 1a, 2a).

After Southern blot hybridization of pLCS, however, a clear hybridization signal was detected only in *Podarcis* and *Algyroides* genera and in *Lacerta dugesi* (Figs 1b, 2b), whereas it was absent in all the studied species belonging to *Lacerta* part I and *Lacerta* part II. All the species of *Podarcis* showed the same hybridization pattern as *P. sicula*, though the hybridization signal at the level of low mol. wt bands (i.e. those corresponding to the monomer and dimer) was fainter. The same pattern as in *Podarcis* was also observed in *Algyroides moreoticus*, whereas in *A. fitzingeri* it was much fainter and lacking at the level of the monomeric unit (Fig. 2b). Finally, in *L. dugesii*, hybridization was positive only in higher mol. wt bands.

In situ hybridization (Fig. 3) confirmed that the Taq I satellite was generally localized at the level of the centromere, as was previously seen in *Podarcis sicula* (Capriglione *et al.*, 1989).

This localization was not limited to the species of *Podarcis*, but it was also present in a species ascribed to a different genus, such as *L. dugesii* (Fig. 2).

Table 2. Analysis of the base composition and variation in the clone pLCS

	Number of bases		Unchanged bases		Changed bases	
	n	%	*n*	%	*n*	%
A	63	33	40	63	23	37
T	45	24	30	67	15	33
C	48	25	18	37	30	63
G	34	18	18	53	16	47

DISCUSSION

The Taq I satellite distribution in the various lacertid species investigated appears of great interest from a phylogenetic standpoint, and provides a valid contribution to the study of the taxonomy of this family, in particular of the so-called *Lacerta* s.l. complex. Recently, this complex has been investigated morphologically, electrophoretically and karyologically (Arnold, 1989; Mayer and Lutz, 1989; Olmo *et al.*, 1989), and several hypotheses have been proposed for its reclassification (Arnold, 1973, 1989; Lutz and Mayer, 1989).

Several opinions are in favor of considering some genera, like *Gallotia* and *Podarcis*, as clearly distinct natural groups, while some doubts exist as to other groups of species like those defined by Arnold (1973), *Lacerta* part I and *Lacerta* part II. Our investigation clearly shows that all the species of the genus *Podarcis* studied have a Taq I satellite DNA which seems to have undergone few variations during the evolution of this genus, all the species exhibiting the same ladder of bands and hybridization pattern. These results confirm that *Podarcis* can be considered as a clearly distinct group among the lacertid lizards.

Our data seem also to provide some interesting phylogenetic information on the relationship between *Podarcis* and other species ascribed to different genera or subgenera, like *Algyroides* or *Lacerta*.

Our results clearly show that *Podarcis* is phyletically distant from the species ascribed to *Lacerta* part II (or *Lacerta* s. str.), like *L. viridis* and *L. lepida*, and from one species included by Arnold (1973) in *Lacerta* part II, like *L. bedriagae*. A greater affinity is instead observed with *Algyroides* and *L. dugesii*.

On the basis of morphological studies, it has been suggested that *L. dugesii* belongs to the same phyletic lineage from which *Podarcis* originated (Richter,

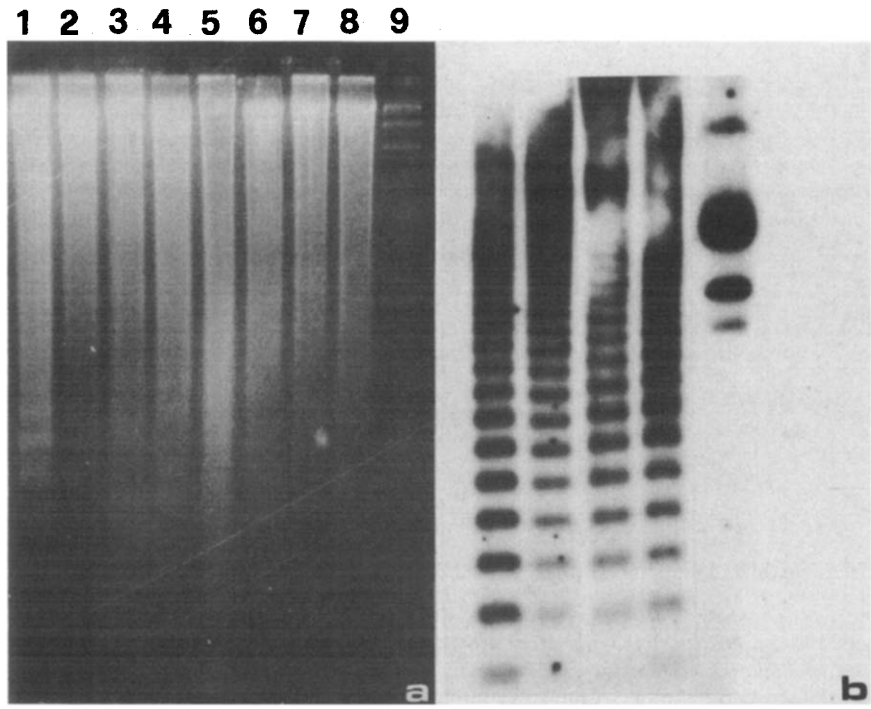

Fig. 1. (a) Nuclear DNAs of different lacertid species treated with the restriction enzyme Taq I: 1) *P. sicula*; 2) *P. muralis*; 3) *P. taurica*; 4) *P. tiliguerta*; 5) *L. dugesii*; 6) *L. bedriagae*; 7) *L. lepida*; 8) *L. viridis*; 9) λ Hind III. (b) Autoradiography of the gel after Southern hybridization with ^{32}P-pLCS.

1979; Arnold, 1989), whereas *Algyroides* would be more distant and would show the same degree of affinity both with *Podarcis* and with the so-called Archeolacertae, a group including several species of *Lacerta* part II, including *L. bedriagae* (Arnold, 1989). Based on electrophoretical and immunological data, Mayer (1989) (pers. commn.), though confirming the resemblance between *L. dugesii* and *Podarcis*, holds however that there is an equally close affinity between the latter genus and *Algyroides*.

Our results agree better with Mayer's data than with the morphological ones. In fact, Southern blotting experiments, though confirming that *L. dugesii* is more closely related to *Podarcis* than to the other

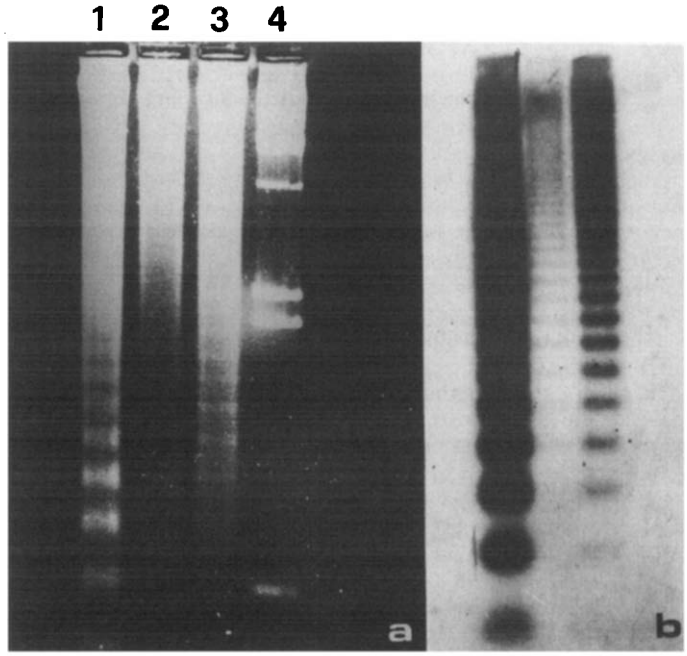

Fig. 2. (a) Agarose gel electrophoresis of the Taq I-digested genomic DNAs of: 1) *P. sicula*; 2) *A. moreoticus*; 3) *A. fitzingeri*; 4) λ Hind III. (b) Autoradiography of the same gel probing with ^{32}P-pLCS.

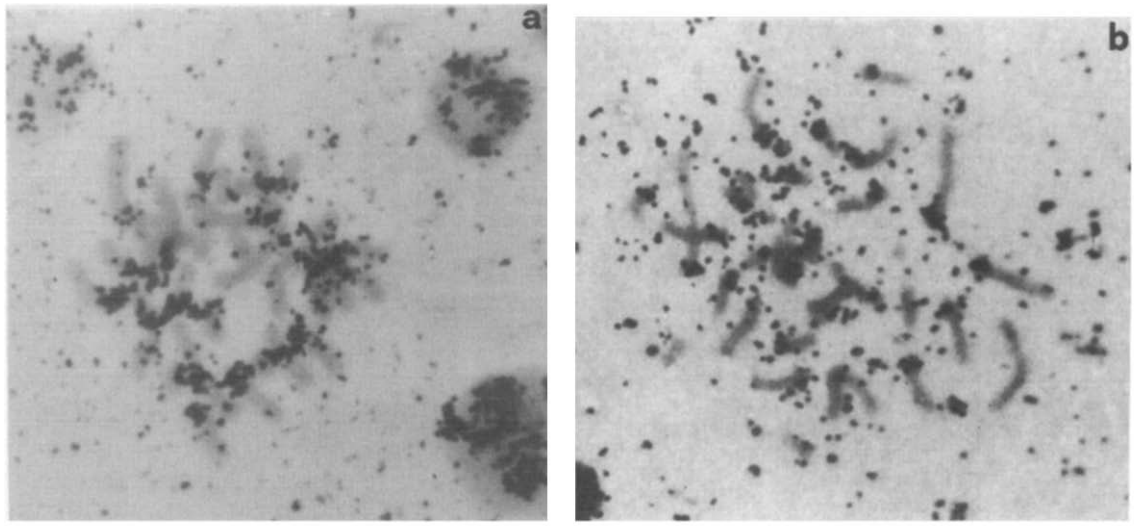

Fig. 3. *In situ* hybridization of ^{3}H-pLCS on mitotic metaphases from bone marrow of (a) *P. sicula* and (b) *L. dugesii*.

species included in *Lacerta* part II, show that *Algyroides* is closer to *Podarcis* than *L. dugesii* is. In fact, in *A. moreoticus*, the hybridization pattern is the same as in the various *Podarcis*, and in *A. fitzingeri*, it is different only in the lack of the band corresponding to the monomeric unit.

However, in *L. dugesii* the Taq I DNA, though being present, differs much more, showing only high mol. wt bands. This clearly shows that this species diverged from the lineage leading to *Podarcis* much earlier than the divergence between the latter and *Algyroides*.

Some characteristics of Taq I satellite DNA lead to the supposition that it is subject to a selective pressure tending to its preservation, and that this has a specific functional meaning. In fact this DNA is relatively ancient, since it can be found in taxa like *L. dugesii* and *Podarcis*, which are supposed to have diverged about 16 million years ago (Mayer and Lutz, 1989). It shows quite a limited variability, as can be seen from the maintenance of the same hybridization pattern not only in the species of the genus *Podarcis* itself, but also in species like *A. moreoticus*, which are ascribed to a different genus (see Arnold, 1973). Lastly, in all the species, this satellite DNA preserves the same localization at the level of centromeric heterochromatin, regardless of the variations in the sequence, as it appears evident by comparing *in situ* hybridization and Southern blotting results in *L. dugesii* and *Podarcis* (Figs 1b, 3).

It has been hypothesized by several authors that some clustered A–T-rich satellite DNAs are involved, directly or through cooperation with particular proteins (Solomon *et al.*, 1986), in the process of heterochromatin condensation. These DNAs are often localized at the level of centromeric or pericentromeric heterochromatin, and their role would depend on the presence of blocks of five or six adenines repeated in phase with the DNA helix. These blocks would determine a stable curvature of the DNA molecule, which would play a relevant role in heterochromatin condensation (Radic *et al.*, 1987; Ng *et al.*, 1986).

Several eukaryotes possess DNA containing adenine stretches preceded by a guanine or cytosine base, and located on centromeric or pericentromeric heterochromatin (Brutlag, 1980; Singer, 1982; Miklos, 1985; Barsacchi Pilone *et al.*, 1986; Lica *et al.*, 1986; Ng *et al.*, 1986; Moyer *et al.*, 1988). Some authors (Radic *et al.*, 1987; de la Torre *et al.*, 1990) have seen that, by treating mouse chromosomes with distamycin A or Hoechst 33258, two substances interacting specifically with these A–T-rich satellite DNAs, heterochromatin condensation drastically decreases.

The Taq I DNA of *Podarcis* also shows frequent adenine stretches preceded by a guanine or cytosine base, which are the most constant portions of the sequence. Its localization on centromeric heterochromatin is also constant in quite distant species.

These observations, though not being conclusive, allow us to hypothesize that the Taq I satellite DNA may have a function, probably related to centromeric heterochromatin condensation.

Acknowledgements—We are grateful to Dr E. Boncinelli for having given us hospitality in his laboratory at the National Research Council (CNR). We also wish to thank Dr D. Acampora for his technical advice on sequencing experiments. This work was supported by a MPI grant.

REFERENCES

Arnold E. N. (1973) Relationships of the Paleartic Lizard assigned to the genera *Lacerta, Algyroides* and *Psammodromus* (Reptilia : Lacertidae). *Bull. Br. Mus. nat. Hist. (Zool.)*, London **25**, 291–366.

Arnold E. N. (1989) Toward a phylogeny and biogeography of the Lacertidae: relationships within an Old-World family of lizards derived from morphology. *Bull. Br. Mus. nat. Hist. (Zool.)*, London **55**, 209–257.

Barsacchi-Pilone G., Batistoni R., Andronico F., Vitelli L. and Nardi I. (1986) Heterochromatic DNA in *Triturus* (Amphibia, Urodela). I. A satellite DNA component of the pericentric C-bands. *Chromosoma* **43**, 435–446.

Brutlag D. L. (1980) Molecular arrangement and evolution of heterochomatic DNA. *A. Rev. Genet.* **14**, 121–144.

Capriglione T., Olmo E., Odierna G., Smith D. I. and Miller O. J. (1989) Genome composition and tandemly repetitive sequence at some centromeres in the lizard *Podarcis s. sicula* Raf. *Genetica* **79,** 85–91.

Gall J. G. and Pardue M. L. (1971) Nucleic acid hybridization in cytological preparations. *Meth. Enzymol.* **XXI-D,** 470–480.

Jeffreys A. J. and Flavell R. A. (1977) A physical map of the DNA flanking the rabbit B-globin gene. *Cell* **12,** 429–439.

Lica L. M., Narayanswami S. and Hamkalo B. A. (1986) Mouse satellite DNA, centromere structure and protein pairing. *J. Cell Biol.* **103,** 1145–1151.

Lima de Faria A., Arnason U., Widegren B., Essen-Mooller J., Isaksson M., Olsson E. and Jaworska M. (1984) Conservation of repetitive DNA sequences in deer species studied by Southern blot transfer. *J. molec. Evol.* **20,** 17–24.

Lutz D. and Mayer M. (1991) Chemosystematische Untersuchugen zur phylogenase der Sammelgattung *Lacerta* (Reptilia: Sauria: Lacertidae). *Z. f. Zool. Syst. Evolutionforsch* (in press).

Miklos G. L. G. (1985) Localized highly repetitive DNA sequences in vertebrate and invertebrate genomes. In *Molecular Evolutionary Genetics* (Edited by MacIntyre R. J.), pp. 241–321. Plenum Press, New York.

Moyer S. P., Ma D. P., Thomas T. L. and Gold J. R. (1988) Characterization of a highly repeated satellite DNA from the cyprinid fish *Notropis lutrensis. Comp. Biochem. Physiol.* **91B,** 639–646.

Ng R., Ness J. and Carbon J. (1986) Structural studies on centromeres in the yeast *Saccharomyces cerevisiae.* In *Extrachromosomal Elements in Lower Eukaryotes* (Edited by Wickner R. B., Hiiebusch A., Lambowitz A., Gunzales I. C. and Hollaender A.), pp. 478–492. Plenum Press, New York.

Olmo E., Odierna G., Capriglione T. and Cardone A. (1989) DNA and chromosome evolution in Lacertid lizards. In *Cytogenetics of Amphibians and Reptiles* (Edited by Olmo E.), pp. 181–204. Birkhauser Verlag, Basel, Boston, Berlin.

Olmo E., Odierna G. and Cobror O. (1986) C-Banding variability and phylogeny of Lacertidae. *Genetica* **71,** 63–74.

Peacock W. J., Appels R., Drensennir P., Lohe A. R. and Gerlach W. L. (1977) Highly repeated DNA sequences: chromosomal localization and evolutionary conservation. In *Int. Cell Biol.* 1976–1977 (Edited by Brinkley B. R. and Parker K. R.), pp. 494–1108. Rockfeller University Press, New York.

Radic M. L., Lundgren K. and Hamkalo B. (1987) Curvature of mouse satellite DNA and condensation of heterochromatin. *Cell* **50,** 1101–1108.

Richter K. (1979) *Lacerta dugesii* Milne–Edwards, 1829 and *Lacerta perspicillata* Dumeril et Bibron, 1938 georfen zur Gattung *Podarcis* Wagler, subgenus *Teira* Gray, 1838 (Reptilia, Lacertidae). *Zool. Abh. Dresden* **36,** 1–9.

Sanger F., Nicklen S. and Coulson A. R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. natn. Acad. Sci. USA* **74,** 5463–5467.

Singer M. F. (1982) Highly repeated sequences in mammalian genomes. *Int. Rev. Cytol.* **76,** 67–112.

Solomon M. J., Strauss F. and Varshavsky A. (1986) A mammalian high mobility growth protein recognizes any stretch of six A–T base pairs in duplex. *Proc. natn. Acad. Sci. USA* **83,** 1276–1280.

Southern E. M. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. molec. Biol.* **98,** 503–517.

Torre J. de la, Goyanes V. and Gosalvez J. (1990) Discontinuous under-condensation of centromeric heterocromatin in mouse chromosomes: evidence in Hoechst 33258-treated cells. *Cytogenet. Cell Genet.* **54,** 55–57.