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A MITOCHONDRIAL DNA RESTRICTION ENZYME CLEAVAGE MAP FOR THE SCORPION *HADRURUS ARIZONENSIS* (IURIDAE)

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Abstract. Mitochondrial DNA (mtDNA) was prepared from a single individual of the scorpion *Hadrurus arizonensis* Ewing. The total size of the mitochondrial genome was estimated to be 13 850 to 14 000 base pairs. The mtDNA was surveyed for cleavage sites using 17 six-base restriction enzymes and three four-base enzymes. The technique of double digests was used to construct a map of the cleavage sites generated in this mtDNA by nine six-base restriction enzymes.

We present a restriction enzyme cleavage map and information on the size and base composition of the mitochondrial genome of the scorpion *Hadrurus arizonensis* Ewing (Iuridae). This is the first published restriction site map for an arachnid mitochondrial genome. This map will be useful in systematic studies of *Hadrurus* and related scorpions, in studies which use mtDNA markers in the study of *H. arizonensis* population biology, and as a guide for cloning and sequencing the *H. arizonensis* mitochondrial genome. Excellent discussions of animal mitochondrial DNA (mtDNA) and its use in systematics, biogeography and population biology can be found in Avise et al. (1987), Brown (1985) and Moritz et al. (1987).

METHODS AND MATERIALS

The scorpions used in this study were collected from Cochise Co., Arizona in June, 1989 by K. and D. Aiken, shipped live to the University of Michigan, and stored at −80° C. MtDNA was prepared from muscle tissue (tail, pedipalps) of one adult using the methods described in Smith and Brown (1990). A second individual has been kept as a voucher specimen. The mtDNA was analyzed by digestion with restriction enzymes. Aliquots of the mtDNA were digested with the six-base and four-base restriction enzymes listed in Table 1, using buffer and temperature conditions recommended by the manufacturers (Bethesda Research Laboratories, Boehringer Mannheim Biochemicals, International Biotechnologies, and New England Biolabs). The resulting fragments were radioactively end-labeled with ^{32}P-deoxynucleotides and separated by electrophoresis on 1% agarose and 4% polyacrylamide gels (Brown 1980; Wright et al. 1983) and visualized by autoradiography. The sizes of the DNA fragments were estimated by comparison with size standards (HindIII and AvaI/BglII digests of wild-type lambda phage DNA and HaeIII digests of phage phi-X174 DNA) run on the same gels. The relative positions of restriction enzyme cleavage sites were mapped by means of double digests (Brown & Vinograd 1974).

RESULTS AND DISCUSSION

The tissues from the tail and one pedipalp of one *H. arizonensis* yielded approximately 1.5 μg of mtDNA. The mitochondrial genome of *H. arizonensis* is small, approximately 13 850 to 14 000 base pairs (13.85 to 14.0 kilobase pairs or kb). Table 1 shows the number of cleavages generated by each restriction enzyme in *H. arizonensis* mtDNA. Figure 1 shows the relative positions of the cleavage sites generated by nine of the enzymes.

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Table 1.—Six-base and four-base restriction endonucleases used in this survey, and the number of cleavages generated by each enzyme in the mitochondrial DNA of *Hadrurus arizonensis*. (A = adenine, T = thymine, C = cytosine, G = guanine, Py = any pyrimidine, Pu = any purine, N = any base.)

Enzyme	Recognition sequence(s)	Number of recognition sites
AflII	CTTAAG	1
AseI	ATTAAT	>10
AvaI	CPyCGPuG	0
BamHI	GGATCC	1
BclI	TGATCA	6
CfoI	GCGC	0
DraI	TTTAAA	>10
EcoO109	PuGGNCCPy	1
EcoRI	GAATTC	3
EcoT22I	ATGCAT	1
HaeIII	GGCC	1
HindII	GTPyPuAC	4
HindIII	AAGCTT	7
MspI	CCGG	0
NdeI	CATATG	2
PvuII	CAGCTG	1
SacI	GAGCTC	0
SpeI	ACTAGT	4
SspI	AATATT	>10
XbaI	TCTAGA	0

These data suggest that the mtDNA of *H. arizonensis*, like that of insects such as *Drosophila* (e.g., Clary & Wolstenholme 1985) and the honey bee (*Apis mellifera* L.; Crozier et al. 1989) is rich in adenine (A) and thymine (T) and poor in guanine (G) and cytosine (C). Digestion with four-base enzymes whose recognition sites contain only cytosines and guanines, reveals one (HaeIII) or no (CfoI, MspI) cleavage sites. At the other extreme, digestion with the 6-base enzymes AseI, DraI and SspI, whose recognition sites contain only A's and T's, revealed large numbers of cleavage sites.

While most animal mtDNA's fall in a rather narrow size range of 16 to 18 kb, the total known size range for animal mtDNA's is from 39 kb in the scallop *Placopecten magellanicus* (Gmelin) (Snyder et al. 1987) to 14.285 kb in the nematode *Ascaris suum* (Wolstenholme et al. 1987). Nearly all animal mtDNA's that have been investigated contain the same genes, coding for 22 transfer RNA's, 13 proteins and two ribosomal RNA's (Brown 1985; Moritz et al. 1987), plus a non-coding control region (the D-loop in vertebrates or AT-rich region of insects and other invertebrates). Variation in the size of animal mtDNA's is commonly due to variation in the size of the control region, which may contain a variable number of tandemly repeated sequences (e.g., Fauron and Wolstenholme 1980; Harrison et al. 1985; Densmore et al. 1985; Solignac et al. 1986) but may also be due to duplications in coding regions (e.g., Moritz & Brown 1986, 1987), or small tandem repeats, inserts and deletions scattered throughout the mitochondrial genome (e.g., Powers et al. 1986). The unusually small mitochondrial genome of the nematodes is lacking the ATPase 8 gene, and the tRNA's have lost a

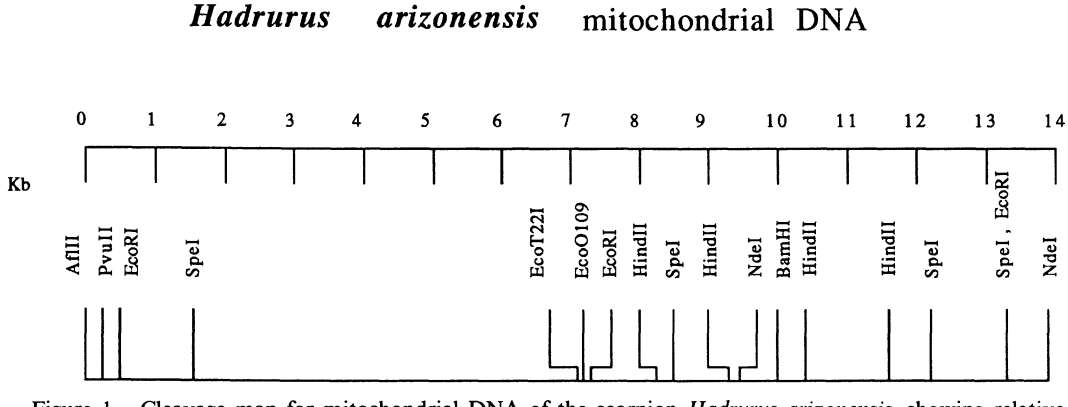

Figure 1.—Cleavage map for mitochondrial DNA of the scorpion *Hadrurus arizonensis*, showing relative positions of the cleavages generated by the enzymes AflII, BamHI, EcoRI, EcoT22I, HindII, NdeI, PvuII, and SpeI. The single AflII site was arbitrarily chosen as the starting point for this linear map of the circular mtDNA molecule. Subsequent sequencing (Smith unpubl. data) shows that the SpeI and EcoRI sites at approximately 13.35 kb are 7 base pairs apart, with the SpeI site preceding the EcoRI site. The scale is in thousands of base pairs, or kilobase pairs (kb).

stem and loop structure. (Wolstenholme et al. 1987). This raises the possibility that the unusually small mitochondrial genome of *H. arizonensis* (13.85–14.0 kb) is also lacking a gene typically found in animal mtDNA. In this regard it is interesting that the mtDNA of other chelicerates, including the horseshoe crab, *Limulus polyphemus* (L.) (Xiphosurida), vinegaroon, *Mastigoproctus giganteus* (Lucas) (Thelyphonida), and the spiders *Anelosimus eximius* (Keyserling) and *A. studiosus* (Hentz) (Araneae: Theridiidae), also have small mitochondrial genomes of 14–15 kb (Smith unpubl. obs.).

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