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Mitochondrial DNA from a spider mite: isolation, restriction map and partial sequence of the cytochrome oxidase subunit I gene

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Abstract

Mitochondrial (mt) DNA of the phytophagous mite *Tetranychus urticae* was purified and a restriction map was constructed. The 12.5 kb long genome is the shortest animal mtDNA known. A 564 bp clone comprising part of the gene for cytochrome oxidase subunit I was sequenced. As has been found in insects, the mitochondrial sequences of mites are extremely A+T rich (75% on average, 96.5% at the third codon position).

Introduction

Despite the economic and medical importance of numerous mite species, the genome of this group is not well characterized. Mites have been examined for allergenic protein-encoding genes (Tovey *et al.*, 1989) and ribosomal DNA sequence polymorphism (Navajas *et al.*, 1992; Wesson & Collins, 1992). The mitochondrial genome, however, remains completely uncharacterized in Acari in general. The scarcity of molecular data probably results from the small size of most mite species and the relative difficulty of obtaining them in large numbers.

In order to get first information on the mitochondrial genome of mites, we isolated mitochondrial DNA (mtDNA) of the phytophagous spider mite *Tetranychus urticae* Koch and constructed a restriction map. We also partially sequenced the cytochrome oxidase subunit I gene (COI) and compared it to sequences from other animals.

Material and methods

Mitochondrial DNA preparation

A long-established laboratory strain of *T. urticae* originating from southern France was used for DNA isolation. Mites were mass-reared on bean plants and harvested from the leaves by brushing. The animals were ground in 1/10 (W/V) 1 M Tris HCl pH8 buffer containing 9% sucrose, 25 mM $CaCl_2$, 0.015% Triton X100 and 1 mM EDTA. Mitochondria were collected by differential centrifugation, resuspended in the same volume of 0.1 M NaCl, 7% sucrose, 1 mM EDTA and lysed by adding 1/5 (V/V) 0.5 M Tris HCl pH 9.2, 125 mM EDTA, 2.3% SDS buffer. The sample was incubated 30 min at 37 °C; deproteinization was achieved by phenol and chloroform extractions and DNA was ethanol precipitated and dissolved in 8 ml of 10 mM Tris-HCl pH 8.0. Closed circular DNA was separated from linear DNA by addition of 8.2 g CsCl and 60 ml 10% bisbenzidine and centrifugation at 42,000 rpm for 48 h. The higher, 2-bisbenzidine band was recovered and the bisbenzimine was extracted with water-saturated n-butanol. DNA was then precipitated by addition of 2.2 volumes of 70% ethanol.

Cloning and sequencing

The purified mtDNA was digested by Sau3A and the fragments were ligated into the BamHI site of Bluescript SK (Stratagene). The clones obtained were sequenced using the chain-termination method (Sanger, Nicklen & Coulson, 1977). Sequences were compared with the *Drosophila yakuba* mitochondrial genome (Clary & Wolstenholme, 1985).

Hybridization
Purified *T. urticae* mtDNA was cleaved with restriction endonucleases, electrophoresed in an agarose gel and transferred onto Hybond N^+ membranes using standard procedures. A clone (pTB 3′) with COI sequences was ^{32}P-labeled using the random primer DNA labeling method (Feinberg & Vogelstein, 1982) and used as a probe. Medium stringency conditions were used for hybridization (0.1% SDS, 6 × SSPE, 55 °C) and washes (0.1% SDS, 2 × SSPE, 55 °C).

Restriction fragment map
Cleavage site positions in the mitochondrial genome were determined using the double-digestion method. One to five nanograms of purified mtDNA were used for each digestion with pair combinations of the six restriction endonucleases: Hind III, Xho I, Spe I, Msp I, Bgl II and Xba I. Restriction fragments were end-labeled according to Drouin (1980). Sizes were resolved by electrophoresis in 0.8% agarose gels. Lambda DNA cut with Hind III was used as a molecular weight standard.

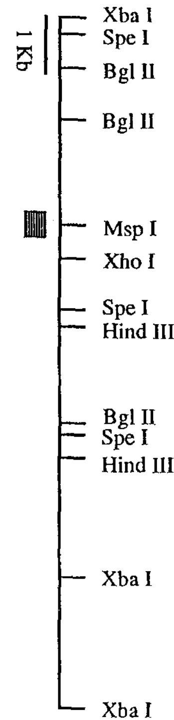

Fig. 1. Restriction map of the mitochondrial genome from the mite *Tetranychus urticae*. The circular genome was linearized at the Xba I site. No Bam HI site was detected. The shaded box marks the position of the clone.

Results and discussion

Characterization of mitochondrial DNA
The restriction map constructed from ultrapurified mtDNA of *T. urticae* is shown in Fig. 1. The total length of the molecule was estimated to be 12.5 Kb. This is the shortest animal mtDNA reported to date. Although mitochondrial genomes of invertebrates have not been extensively investigated, it is interesting to note that the smallest mitochondrial molecule reported (13.79 Kb) is from the nematode *Caenorhabditis elegans* (Okimoto *et al.*, 1992). Among the arachnids only the mtDNA of a scorpion has been analyzed and the total size is 13.8 to 14 Kb (Smith & Brown, 1991). Nearly all known mitochondrial genomes from animals contain the same genes, and length differences are due either to size variation of the non-coding regions or to deletions and insertions spread over the genes. However, the possibility that one or a few of the genes normally found in animal mtDNA are absent from the mtDNA of mites cannot be ruled out. The ATPase 8 gene is not located in the mtDNA of nematode worms. Moreover the nematode mitochondrial genome carries unusually short tRNA genes (Wolstenholme *et al.*, 1987). An alternative explanation for the economy of size of the mite mitochondrial genome is that both DNA strands carry encoding genes. In some totally sequenced metazoan mtDNA, different portions of protein and tRNA genes are transcribed in opposite strands of the molecule. Nevertheless, overlaps concern only a few nucleotides (Wolstenholme, 1992). Further mite studies will determine whether the gene content and organization of mtDNA are conserved in the Acari.

Cytochrome oxidase subunit I sequence
Several clones were found to be homologous to the sequence of the gene for subunit I of the cytochrome oxidase of *Drosophila yakuba* (Clary & Wolstenholme, 1985). A 564 base pair (bp) cloned fragment was sequenced (EMBL accession number X74571). The position of the cloned fragment on the restriction map is shown on Fig. 1, and was determined by using it as a probe against blots of restricted mite mtDNA (not shown). Base composition of the mite COI sequence is dependent on codon position, the sequence being overall extremely rich in A+T (average 75%). The bias is particularly pronounced in third-codon positions (96.5%). Similar compositional biases are also known to prevail in insects (Clary & Wolstenholme, 1985;

Crozier & Crozier, 1993) as well as in another studied arachnid (Smith & Brown, 1991). The inferred mite amino acid sequence for the partial COI analysed has 24.6%, 26.2%, and 30.5% of amino acid replacements compared to *Drosophila*, *Xenopus*, and Sea Urchin, respectively.

Although only a partial region of mite mtDNA was sequenced, this information should make possible the design of mite-specific oligonucleotide probes allowing polymerase chain reaction amplifications in other mite species. Such data should be useful to investigate diversity in Tetranychidae mites and to address taxonomical and evolutionary questions in this group of arthropods.

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