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SHORT NOTE

Mitochondrial COI sequences in mites: evidence for variations in base composition

M. Navajas,[1,2] D. Fournier,[3] J. Lagnel,[1,2] J. Gutierrez[1] and P. Boursot[4]

[1]*Laboratoire de Zoologie, INRA-ENSAM-ORSTOM, Montpellier,* [2]*Institut des Sciences de l'Evolution (CNRS, UMR-5554), Laboratoire Génétique et Environnement, Université Montpellier II,* [3]*Laboratoire d'Entomologie, Université Paul Sabatier, Toulouse,* [4]*Laboratoire Génome et Populations (CNRS, UPR 9060), Université Montpellier II, France*

Abstract

Studies of mitochondrial DNA sequences in a variety of animals have shown important differences between phyla, including differences in the genetic codes used, and varying constraints on base composition. In that respect, little is known of mites, an important and diversified group. We sequenced a portion (340 nt) of the cytochrome oxidase subunit I (COI) encoding gene in twenty species of phytophagous mites belonging to nine genera of the two families Tetranychidae and Tenuipalpidae. The mitochondrial genetic code used in mites appeared to be the same as in insects. As is generally also the case in insects, the mite sequences were very rich in A + T (75% on average), especially at the third codon position (94%). However, important variations of base composition were observed among mite species, one of them showing as little as 69% A + T. Variations of base composition occur mostly through synonymous transitions, and do not have detectable effects on polypeptide evolution in this group.

Keywords: COI, mitochondrial genetic code, Tetranychidae, spider mites, base composition.

Received 25 March 1996; accepted 10 June 1996. Correspondence: Dr Maria Navajas, Laboratoire de Zoologie, INRA, 2 Place P. Viala, 34060 Montpellier, France.

Introduction

There has been considerable interest in mitochondrial DNA (mtDNA) in evolutionary studies and its organization and sequence have been determined in a number of animal species (Wolstenholme, 1992, and references therein). This has led to the discovery that several genetic codes differing from the so-called universal code are used in animal mitochondria, such as the code of vertebrates, echinoderms, insects, nematodes, platyhelminths and cnidaria (Osawa *et al.*, 1992; Wolstenholme, 1992). Comparative studies of different species have revealed some major trends of the mode of evolution of mtDNA in different groups (Moritz *et al.*, 1987). Although base composition can vary from one gene to another, gross overall differences exist between groups of organisms. For instance, insect mtDNA is overall much more A + T rich than vertebrate mtDNA (Clary & Wolstenholme, 1985; Crozier & Crozier, 1993; Irwin *et al.*, 1991; Jermiin & Crozier, 1994). It is not yet clear how frequent variations in base composition can be, and at what time scale they occur, and additional data are needed at various evolutionary scales.

Although mites are a highly diversified group, with 388 families described, and include many species of medical and agronomic importance, little is known of the organization and mode of evolution of their mtDNA. It is a general feature of mtDNA in animals to be extremely compact, and we have previously found the mitochondrial chromosome of the mite *Tetranychus urticae* to be among the shortest (Fournier *et al.*, 1994). In the present study, part of the mitochondrial Cytochrome Oxidase subunit I (COI) encoding gene was sequenced in twenty species of phytophagous mites belonging to the families Tetranychidae and Tenuipalpidae. These data were used to infer the mitochondrial genetic code in mites and reveal variations of their mtDNA sequence composition.

Results and Discussion

Sequence variation

A total of 340–390 base pair (bp) of the central part of the mitochondrial COI gene was sequenced from the twenty mite species listed in Table 1 (EMBL database accession numbers X74571, X79901 and X80856–X80873). The number of nucleotide differences in pairwise comparisons of species ranged from eighteen (5%) to eighty-six (25%) over the 340 nucleotide region sequenced in all species.

Base composition

Table 1 shows the base composition of the sense strand at the three codon positions in all species. The sequences are overall extremely rich in A+T (average 75%). The first codon positions have more A than T, whereas the second have more T than A. Base composition bias is particularly pronounced at the third codon position, which contains an average 94% A+T. Using Fisher's exact probability test, significant heterogeneity in base composition among species is detected at the third codon position ($P = 0.0003$), but not at the first and second positions. In fact it can be seen in Table 1 that *Eurytetranychus buxi* and, to a lesser extent, *Bryobia kissophila*, have more G+C at the third codon position than the other species. When *E. buxi* is removed from the analysis, the third codon position is no longer significantly heterogenous ($P = 0.285$), so that the higher G+C content of this species is significant, whereas that of *B. kissophila* is not.

The biases in base composition appear very similar in mites and insects, with a high proportion of A+T, specially at the third codon position. Similarly, the A+T content of mitochondrial 16S sequences is 74% in ixodid mites (Black & Piesman, 1994). A related shared characteristic between mites and insects is the rarity of C in first codon position and of G in third. However, variations around this general tendency are observed in insects. Based on known cytochrome oxydase II sequences, Jermiin & Crozier (1994) show that in insects the Endopterygota have a generally higher A+T content (~75%) than other insect divisions (~69%). However, inside the Endopterygota the honeybee *Apis mellifera* (Hymenoptera, Apidae) was found to have a higher A+T content than average (80.0%), whereas the reverse was found in one Coleoptera, Curculionidae (*Sitophilus granarius* 69.8%). On the basis of cytochrome b sequences, one ant, *Tetraponera rufonigra* (Hymenoptera, Formicidae), was found to have reduced A+T content (69.9%) when compared to the other insects studied (73.9–80.7%). Thus, provided these two different genes represent the same general trends on the whole mtDNA molecule, it appears that, among Endopterygota insects, the pressure toward high A+T content

Table 1. Base composition in a *COI* fragment of twenty mite species at the three codon positions.

Species	First				Second				Third			
	A	T	G	C	A	T	G	C	A	T	G	C
Tetranychus urticae	40.7	28.3	24.8	6.2	19.5	44.2	15.9	20.4	37.2	59.3	0.9	2.7
Tetranychus kanzawai	42.5	26.5	24.8	6.2	19.5	44.2	15.9	20.4	38.9	54.9	0.9	5.3
Tetranychus mcdanieli	41.6	26.5	24.8	7.1	19.5	44.2	15.9	20.4	41.6	57.5	0	0.9
Tetranychus pacificus	42.5	25.7	23.9	8	20.4	44.2	15.9	19.5	38.9	54.9	1.8	4.4
Tetranychus gloveri	41.6	26.5	24.8	7.1	17.7	44.2	17.7	20.4	38.9	56.6	2.7	1.8
Tetranychus neocaledonicus	41.6	26.5	24.8	7.1	19.5	43.4	15.9	21.2	42.5	52.2	3.5	1.8
Tetranychus viennensis	40.7	26.5	25.7	7.1	18.6	45.1	15.9	20.4	38.9	60.2	0.9	0
Oligonychus gossypii	40.7	27.4	25.7	6.2	18.6	45.1	15.9	20.4	44.2	50.5	1.8	3.5
Oligonychus platani	41.6	28.3	23.9	6.2	18.6	44.2	16.8	20.4	36.3	54.9	1.8	7.1
Oligonychus ununguis	41.6	27.4	23.9	7.1	18.6	44.2	16.8	20.4	31.9	61.9	3.5	2.7
Eotetranychus carpini	41.6	27.4	23.9	7.1	17.7	45.2	15.9	21.2	36.3	60.2	1.8	1.8
Eotetranychus coryli	42.5	26.5	23	8	17.7	45.2	15.9	21.2	32.7	62.8	1.8	2.7
Eotetranychus tiliarium	43.4	26.5	23.9	6.2	16.8	45.2	16.8	21.2	38.1	56.6	0.9	4.4
Panonychus ulmi	41.6	25.7	25.7	7.1	17.7	45.1	16.8	20.4	38.1	56.6	1.8	3.5
Panonychus citri	40.7	26.5	25.7	7.1	18.6	44.2	15.9	21.3	38.1	55.8	3.5	2.7
Mononychellus progresivus	40.7	29.2	23.9	6.2	18.6	44.2	15.9	21.3	35.4	59.3	0.9	4.4
Eurytetranychus buxi	38.1	29.2	26.5	6.2	15.9	46	16.8	21.3	25.7	52.2	9.7	12.4
Petrobia harti	39.8	25.7	28.3	6.2	15.9	46	15.9	22.2	43.4	55.8	0	0.9
Bryobia kissophila	33.6	30.1	30.1	6.2	15.9	45.1	16.8	22.2	31.9	55.8	5.3	7.1
Cenopalpus pulcher	33.6	35.4	23	8	15	46	16.8	22.2	29.2	68.1	1.8	0.9
Mean	40.5	27.6	25	6.9	18	44.8	16.3	20.9	36.9	57.3	2.3	3.5
Standard deviation	2.5	2.2	1.7	0.6	1.4	0.7	0.5	0.7	4.6	4.0	2.1	2.8
Base-compositional bias		0.24					0.26			0.59		

Base-compositional bias is calculated as: $B = (2/3)\sum_{i=1}^{4}|b_i - 0.25|$ where B is the compositional bias and b_i is the frequency of the ith base.

has been relaxed in at least two lineages leading to one weevil (Coleoptera) and one ant (Hymenoptera), respectively. Our data present an additional case of such a phenomenon in mites, with the A + T content of

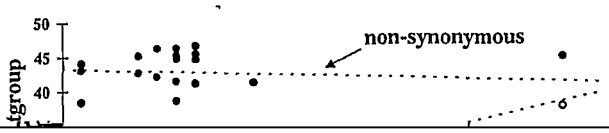

Table 2. Inference of the mitochondrial genetic code used in mites. Numbers correspond to the nucleotide position in the sequence of the mite *Tetranychus urticae* (EMBL accession number 74571).

	ATA						TGA			AGA		AAA		
	85	97	142	157	196	307	130	235	286	43	232	58	61	223
Tetranychus urticae	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGT	AGT	AAA	AAA	AAA
Tetranychus kanzawai	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGT	AGT	AAA	AAA	AAA
Tetranychus mcdanieli	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGA	AGA	AAA	AAA	AAA
Tetranychus pacificus	ATA	ATA	ATA	ATA	ATA	ATG	TGA	TGA	TGA	AGA	AGT	AAA	AAA	AAA
Tetranychus gloveri	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGT	AGA	AAA	AAA	AAA
Tetranychus neocaledonicus	ATA	ATA	ATG	ATA	ATA	ATA	TGA	TGG	TGA	AGA	AGT	AAA	AAA	AAA
Tetranychus viennensis	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGT	AGA	AAA	AAA	AAA
Oligonychus gossypii	ATA	ATA	ATA	ATA	ATA	ATA	TGG	TGA	TGA	AGA	AGT	AAA	AAA	AAG
Oligonychus platani	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGG	TGA	AGC	AGA	AAA	AAA	AAA
Oligonychus ununguis	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGA	AGA	AAA	AAA	AAG
Eotetranychus carpini	ATA	ATA	ATA	ATA	ATA	ATA	TGG	TGA	TGA	AGA	AGT	AAA	AAA	AAA
Eotetranychus coryli	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGA	AGT	AAA	AAA	AAA
Eotetranychus tiliarium	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGT	AGT	AAA	AAA	AAA
Panonychys ulmi	ATA	ATA	ATA	ATA	ATA	ATA	TGA	TGA	TGA	AGA	AGA	AAA	AAA	AAA

Experimental procedures

Amplification and sequencing

A detailed description of the origin of the biological material (listed in Table 1) is presented in Navajas *et al.* (1996). PCR primers were designed from the sequence of a fragment of *Tetranychus urticae* mtDNA COI that we previously cloned (Fournier *et al.*, 1994). The primers were 5' TGATTTTTTGGTCACCCAGAAG 3' and 5' TACAGCTCCTATAGATAAAAC 3', and were used for both DNA amplification and direct sequencing of PCR products. Protocols are described in Navajas *et al.* (1996).

Data analysis

The number of synonymous and non-synonymous substitutions between pairs of sequences were estimated using the program MEGA (v. 1.0) (Kumar *et al.*, 1993). Fisher's exact probabilities were estimated using the Markov chain method described by Guo & Thompson (1992) implemented in the Genepop (v. 1.2) software (Raymond & Rousset, 1995).

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