Heredity **80** (1998) 742–752 *Received 16 June 1997*

Species-wide homogeneity of nuclear ribosomal *ITS2* sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial *COI* polymorphism

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We compared patterns of intraspecific polymorphism of two markers with contrasted modes of evolution, nuclear ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA), in the phytophagous mite *Tetranychus urticae* Koch. The second internal transcribed spacer (ITS2) of rDNA and a fragment in the mtDNA gene coding for Cytochrome Oxidase I (*COI*), were PCR-amplified and sequenced in samples of various geographical origins distributed worldwide. The 15 *COI* haplotypes found fell into two major phylogenetic lineages differing by an average of 5% nucleotide divergence. Samples from the Mediterranean basin were represented in both lineages, and showed no phylogeographical structure. The other samples, from temperate regions of the northern hemisphere, were clustered in one of the lineages and displayed little variation, indicating a recent colonization of this region. In contrast, no variation at all was found at the ITS2 in this species. We sequenced both *COI* and ITS2 in four other species of the genus *Tetranychus* and found that, despite the absence of intraspecific polymorphism, ITS appears to evolve 2.5 times faster than *COI*. We argue that rDNA homogeneity over the species range of *T. urticae* results from the high colonization potential of this species, preventing long-term differentiation. Preliminary data on two other mite species (*Amphitetranychus viennensis* Zacher and *Mononychellus progresivus* Doreste) with stricter ecological requirements and more restricted colonization potential revealed substantial and concordant geographical differentiation for both ITS2 and *COI*.

Keywords: cytochrome oxidase I (*COI*), internal transcribed spacer (ITS2), intraspecific variation, phylogeography, tetranychid mites.

Introduction

A major challenge in evolution is to infer evolutionary processes from the observation of patterns of variation. Because different regions of the genome can have different modes of evolution and/or transmission, they can be affected differently by evolutionary forces in natural populations, and thus convey complementary and/or contrasting information about the evolutionary history of the species. In fact, two of the most popular markers used in molecular evolution, mitochondrial DNA (mtDNA) and nuclear ribosomal DNA (rDNA), have quite different properties, which could translate into different consequences of mutation, drift, migration and selection on patterns of geographical variation and molecular divergence. The patterns of evolution of animal mtDNA and its performance as a marker of intraspecific variation are well understood (Avise, 1994; Simon *et al*., 1994). Nuclear ribosomal DNA has proven very useful in inferring species phylogenies at various evolutionary scales (Hillis & Dixon, 1991). Despite rDNA being present in multiple copies in the genome, its use for that purpose is rendered possible because of the process of concerted evolution (Dover, 1982), ensuring intra-

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specific diversity to be generally reduced as compared to interspecific divergence. This property could apply to intraspecific variation, and geographical isolation between populations could lead to the fixation of alternative rDNA variants. Conversely, mixing of differentiated populations could lead to transient inter- and/or intraindividual heterogeneity. Studies of intraspecific polymorphism of the fast evolving internal transcribed spacers (ITS) have revealed different patterns of rDNA variation depending on the species. For instance, among arthropods, very low variation of the consensus sequences of individuals sampled over the whole species area was reported in Anopheline mosquitoes (Fritz *et al*., 1994). Low intraspecific variation has also been found in two tick species (McLain *et al*., 1995), in *Drosophila* (Schlötterer *et al*., 1994) and in strains of the *Nasonia* species complex (Campbell *et al*., 1993). However, substantial intraindividual heterogeneity of ITS sequences has been reported in two species of mosquitoes (Wesson *et al*., 1993), in blackflies (Tang *et al*., 1996) and in a beetle (Vogler & DeSalle, 1994). It has been suggested that this extreme intraindividual variability, of the same order as interindividual variability, resulted from the fact that the species were subdivided into races, ecotypes or subspecies that had distinct histories, but between which a certain amount of secondary exchange occurred and fuelled the mixing of differentiated ITS sequences, delaying or preventing homogenization (Vogler & DeSalle, 1994; Tang *et al*., 1996; Campbell *et al*., 1997). More studies of this type are needed, however, before any generalization can be made about the influence of geographical differentiation and gene flow on the degree of intraspecific homogeneity of rDNA.

One way to address the question of the influence of geographical isolation is to study in the same species both mtDNA and rDNA variation, using the former to infer historical patterns of gene flow. In this paper we study geographical variation of the mitochondrial cytochrome oxidase I gene (*COI*) and the second nuclear ribosomal internal transcribed spacer (ITS2) in the spider mite *Tetranychus urticae* Koch. This species is a cosmopolitan pest injurious to many crops. The mite is highly polyphagous, has been reported to occur on more than 900 different host plants and has been recorded in about 100 countries, with higher prevalence in areas with a temperate or Mediterranean climate. We present data showing the extent of ITS and mtDNA variation in this species. We also present preliminary data on the intraspecific variation of the two markers in two other mite species with greater specificity and ecological requirements. In order to interpret the differences observed between the patterns of intraspecific variation of the two markers, we also compared their rates of evolution between five different species of the same genus.

Materials and methods

Collection of mites

Specimens of *T. urticae* were collected from several host plants in 18 different localities from 12 different countries in the northern hemisphere between parallels 30 and 57 (Fig. 1 and Table 1). Samples include both green and red mites; these colours result from different haemolymph pigments (De Boer, 1985). Four other *Tetranychus* species (*T. kanzawai* Kishida, *T. mcdanieli* McGregor, *T. pacificus* McGregor and *T. neocaledonicus* André, Table 1) were analysed to assess the relative sequence divergence rate of the two markers studied. Several populations of two other tetranychid mites were also investigated (Table 1): two sites in Japan and four in France in the case of *Amphitetranychus viennensis* and two sites in South America and two in Africa for *Mononychellus progresivus.* Sequences of *M. progresivus* mites have been presented in a previous study (Navajas *et al*., 1994). In most cases animals caught in the field were analysed; in some cases we analysed their descendants born in the laboratory (Table 1).

DNA extraction

Total DNA was extracted from individual adult females. The material was either fresh, frozen or preserved in 100% ethanol. Ethanol-preserved specimens were rinsed for 10 s in 20 μL of water three times prior to DNA extraction. The mite was crushed at 60°C with a plastic pestle in a 1.5 mL microcentrifuge tube containing 200 μL extraction buffer (2% CTAB, 1.4 M NaCl, 0.2% 2-b mercaptoethanol, 20 mM EDTA, 100 mM TRIS-HCl pH 8.0). The tube was incubated at 60°C for 5–30 min. Proteins were removed with one volume chloroform/isoamyl alcohol. DNA was precipitated with one volume isopropanol, the pellet was washed with ethanol (76% v/v containing 10 mM of ammonium acetate) and resuspended in 20 μL of double distilled water. Two or 4 μL were used as a PCR template. For some samples (*T. kanzawai*, *T. mcdanieli*, *T. pacificus*, *T. neocaledonicus and M. progresivus*), a rapid extraction protocol based on the chelating resin Chelex 100 was used as previously described in Navajas *et al*. (1996).

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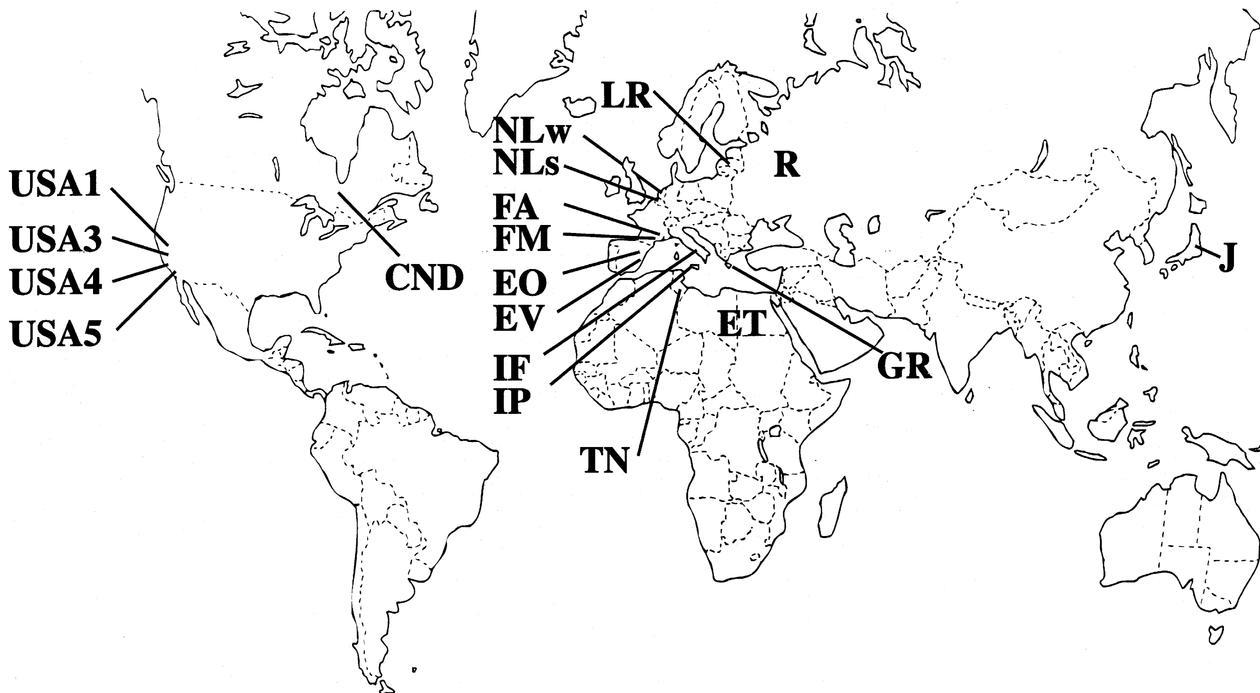

Fig. 1 Map of the sampling localities of the spider mite, *Tetranychus urticae*. The codes for each location are as in Table 1.

PCR amplification and sequencing

Two target DNA fragments were PCR-amplified and sequenced: the complete ribosomal ITS2 and a fragment in the central part of the mitochondrial *COI* gene. The amplification reactions were carried out in a 50 μL volume containing 2 units of Taq polymerase (Eurogentec, Belgium), 1 × enzyme buffer supplied by the manufacturer, 35 pmoles of each primer, 5 nmoles of each dNTP, 75 nmoles of $MgCl_2$ and 2 μL DNA as matrix in *ITS2* amplification and 4 μL DNA in *COI* amplification. After an initial denaturation step at 95°C for 4 min, the reaction consisted of 35 cycles with 1 min at 92°C, 1 min at 51°C and 1.5 min at 72°C. The PCR products were purified using glass beads (GeneClean II Kit, Bio 101, USA). One-third of the eluted volume was used for sequencing with a Sequenase 2.0 kit (US Biochemical Corp.) following the manufacturer's instructions and using ^{35}SdATP as label. We produced full sequences of both strands for one specimen from each origin of each species studied. Other specimens were sequenced for only one strand.

We used the *COI* primers previously designed specifically for tetranychid mites (Navajas *et al.*, 1996). They were 5′-TGATTTTTTGGTCACCCAGAAG-3′ and 5′-TACAGCTCCTATAGATAAAAC-3′. The ITS2 was amplified using the primers 5′-ATATGCTTAAATTCAGCGGG-3′ and 5′-GGGTCGATGAAGAACGCAGC-3′. These primers were defined in the highly conserved 5.8S and 28S flanking regions as previously published in Navajas *et al.* (1994) and subsequently modified to improve specificity for tetranychids. The same PCR primers were used for sequencing. Two additional primers were used for sequencing the ITS2 molecule: 5′-CGACTTTAGCGTCGTCAGAT-3′ for all species and 5′-TATCTAGATCATGGGAAAT-3′ for *M. progresivus.*

Sequence analyses

Phylogenetic analysis of the *COI* sequences was performed with several programs included in the PHYLIP 3.5c package (Felsenstein, 1993). Divergences between sequences were estimated using the program DNADIST, with the Kimura two parameter distance option (Kimura, 1980) and a transition/transversion ratio of 2.0. A tree was built using the Neighbour Joining method (program NEIGHBOR). The same analyses were also performed on 1000 bootstrapped datasets generated by the program SEQBOOT. A majority rule consensus tree was determined from the resulting 1000 trees, using the program CONSENSE. The program MEGA 1.0 (Kumar

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Table 1 Collection sites of mites

Species	Abbreviation	Location	Country	Host plant	N		Mite
					COI	*ITS2*	Colour
Tetranychus urticae	ET	Cairo	Egypt	*Convolvulus arvensis*	1	1	G
	TN	Sousse	Tunisia	*Malva* sp.	1	1	R
	GR	Egion	Greece	*Citrus limon*	1	1	R
	IF	Ferrara	Italy	*Viola* sp.†	3	3	R
	IP	Palermo	Italy	*Citrus limon*	1	1	R
	EO	Onda	Spain	*Citrus reticulata*	1	1	R
	EV	Valencia	Spain	*Citrus aurantium*	1	0	R
	FM	Montpellier	France	*Viola* sp.	3	3	R
	FA	Antibes	France	*Phaseolus* sp.	1	3	G
	NLs	Amsterdam	The Netherlands	*Sambucus* sp.†	3	3	G (wild strain)
	NLw	Amsterdam	The Netherlands	*Sambucus* sp.†T	3	3	G (white eye strain)
	LR	Riga	Latvia	*Rosa* sp.	2	2	G
	R	Moscow	Russia	*Phaseolus* sp.*	1	1	G
	CND	Vineland ON	Canada	*Phaseolus* sp.*	0	2	G
	USA1	Davis CA	USA	*Phaseolus* sp.*	1	1	G
	USA3	Irvin CA	USA	*Fragaria* sp.	0	1	G
	USA4	Watsonville CA	USA	*Fragaria* sp.	0	1	G
	USA5	Oxnard CA	USA	*Fragaria* sp.	0	1	G
	J	Ibaraki	Japan	*Pyrus pyrifolia*	1	1	G
Tetranychus kanzawai	—	Brazzaville	Congo	*Manihot esculenta*	1	1	—
Tetranychus mcdanieli	—	Verzenay	France	*Vitis vinifera*	1	1	—
Tetranychus pacificus	—	Davis	USA	*Vitis vinifera*	1	1	—
Tetranychus neocaledonicus	—	Cotonou	Benin	*Vigna unguiculata*	1	1	—
Anphitetranychus viennensis	AvFT	Toulouse	France	*Prunus domestica*	0	1	—
	AvFL	Lozere	France	*Prunus domestica*	1	2	—
	AvFV	Villeneuve/lot	France	*Prunus domestica*	1	1	—
	AvFM	Montpellier	France	*Malus domestica*	3	3	—
	AvJy	Ami, Ibaraki	Japan	*Prunus yedoensis*	3	3	—
	AvJa	Ami, Ibaraki	Japan	*Prunus avium*	1	1	—
Mononychellus progresivus	MpBen	Cotonou	Benin	*Manihot esculenta*	5	4	—
	MpCon	Brazaville	Congo	*Manihot esculenta*	4	4	—
	MpCal	Cali	Colombia	*Manihot esculenta*	5	4	—
	MpBraz	Petrolina	Brazil	*Manihot esculenta*	2	2	—

—, not applicable.
*The original host plant is unknown and the strain was reared on bean leaves.
†The host plant is indicated although the strain was reared for several generations on bean leaves.
Mite colour: G, Green; R, Red.
N indicates how many individuals have been analysed for each molecule.

et al., 1993) was used to calculate distances between species of *Tetranychus* and their standard errors.

The sequences are deposited in the EMBL database under the following accession numbers: X77901, X80855–59, X80861 and X99873–5 for the *COI* sequences and X79902 and X99876–83 for the *ITS2* sequences.

Results

Interspecific ribosomal ITS2 sequence variation

The complete ITS2 and portions of the flanking 3′ end of the 5.8S and the 5′ end of the 28S were PCR-amplified and sequenced in five species of the genus *Tetranychus*, as well as in *A. viennensis* and *M. progresivus*. The boundaries of ITS2 were determined using the conserved sequence of the two flanking regions (5.8S and 28S). Alignments of sequences among *Tetranychus* species implied only very short insertions/deletions and did not pose any problem. Alignment between the different genera was attempted using secondary structure inference (not shown) and is presented in Fig. 2. Its quality is irrelevant because we did not use any intergeneric comparisons in the present study.

The length of the ITS2 sequence was ≅480 base pairs (bp) in the *Tetranychus* species examined, and was shorter in *A. viennensis* (445 bp) and longer in *M. progresivus* (805 bp). One insertion of 283 bp located at position 201 in the sequence of *M. progresivus* accounts for much of its additional length. This is the longest ITS2 recorded for arthropods. The length of the ITS2 of the other tetranychids examined here falls within the range of the known size of ITS2 in arthropods: ≅280 in the aphid *Acyrthosiphon pisum* (Kwon & Ishikawa, 1992); ≅430 in the wasp *Nasonia* (Campbell *et al*., 1993); ≅353–408 in *Drosophila* species (Schlötterer *et al*., 1994), from ≅360 bp (Fritz *et al*., 1994) to ≅430 bp (Porter & Collins, 1991) in *Anopheles* mosquitoes and 678–728 bp in *Ixodes* species (Wesson *et al*., 1993).

The G+C contents in the ITS2 sequences do not vary significantly between tetranychid species (χ^2 nonsignificant) and range from 31.2% for *T. urticae* to 39% for *M. progresivus*. These constitute intermediate values of G+C content with respect to other arthropods sequenced: 20–21.5% in *Drosophila* (Schlötterer *et al*., 1994); 55.3–59% in culicid mosquitoes (Wesson *et al*., 1992; Fritz *et al*., 1994) and 46.7–47.2% in ticks (McLain *et al*., 1995). The percentage of G+C is higher in the sequenced 5.8S fragment of tetranychid species (50.5–53.8%), than in the *ITS2* sequences ($\chi^2_6 = 42$, $P < 0.01$).

We calculated the pairwise nucleotide divergence among five *Tetranychus* mite species and the distance matrix appears in Table 2a. In pairwise comparisons, we have excluded positions with a gap in one of the two sequences. Nucleotide divergences ranged from 1.3% between *T. urticae* and *T. kanzawai*, to 14.1–16.1% between *T. neocaledonicus* and all the other species.

Interspecific COI *vs.* ITS2 *divergence*

Table 2b shows the *COI* pairwise nucleotide divergences of the five *Tetranychus* species studied, based on the 390 bp fragment sequenced (data from Navajas *et al*., 1996). Divergence is 6.2% between *T. urticae* and *T. kanzawai*, and ranges from 10.2 to 11.6% between *T. neocaledonicus* and the other species.

Figure 3 allows the comparison of the ITS2 and *COI* divergences between pairs of *Tetranychus* species. It can be seen that *COI* divergence is higher than that of ITS2 at low divergences, comparable at intermediate divergences (around 8%), and lower at higher divergences (around 15% ITS divergence). The slope of the regression between *ITS2* and *COI* divergence indicates that ITS2 evolves about 2.5 times faster than *COI*. Note that the intercept of the regression of *COI* against ITS2 crosses the *COI* axis at 5% divergence, meaning that zero ITS2 divergence corresponds to an expected *COI* divergence of ≈5% (see Discussion).

Intraspecific variation of ITS2 sequences

The complete ITS2 sequence of 30 individuals of *T. urticae* from 18 localities described in Fig. 1 and Table 1 was determined. Intrapopulation variation was investigated by sequencing two to three individuals in seven of the sampled localities (IF, FM, FA, NLs, NLw, LR and CDN; Table 1). All 30 individuals examined for *T. urticae* had the ITS2 sequence shown in Fig. 2.

The lack of intraspecific variation of the ITS2 sequences in *T. urticae* contrasts with the results obtained for the two other mite species, although the latter were sampled much less intensively. Among the four different geographical origins of *M. progresivus*, the Brazilian individuals were different (by two transversions) from those of all other origins: Colombia, Congo and Benin. There were 10 differences (two transversions, seven transitions and one 3 bp insertion/deletion) between the ITS2 sequences of *A. viennensis* strains from France and

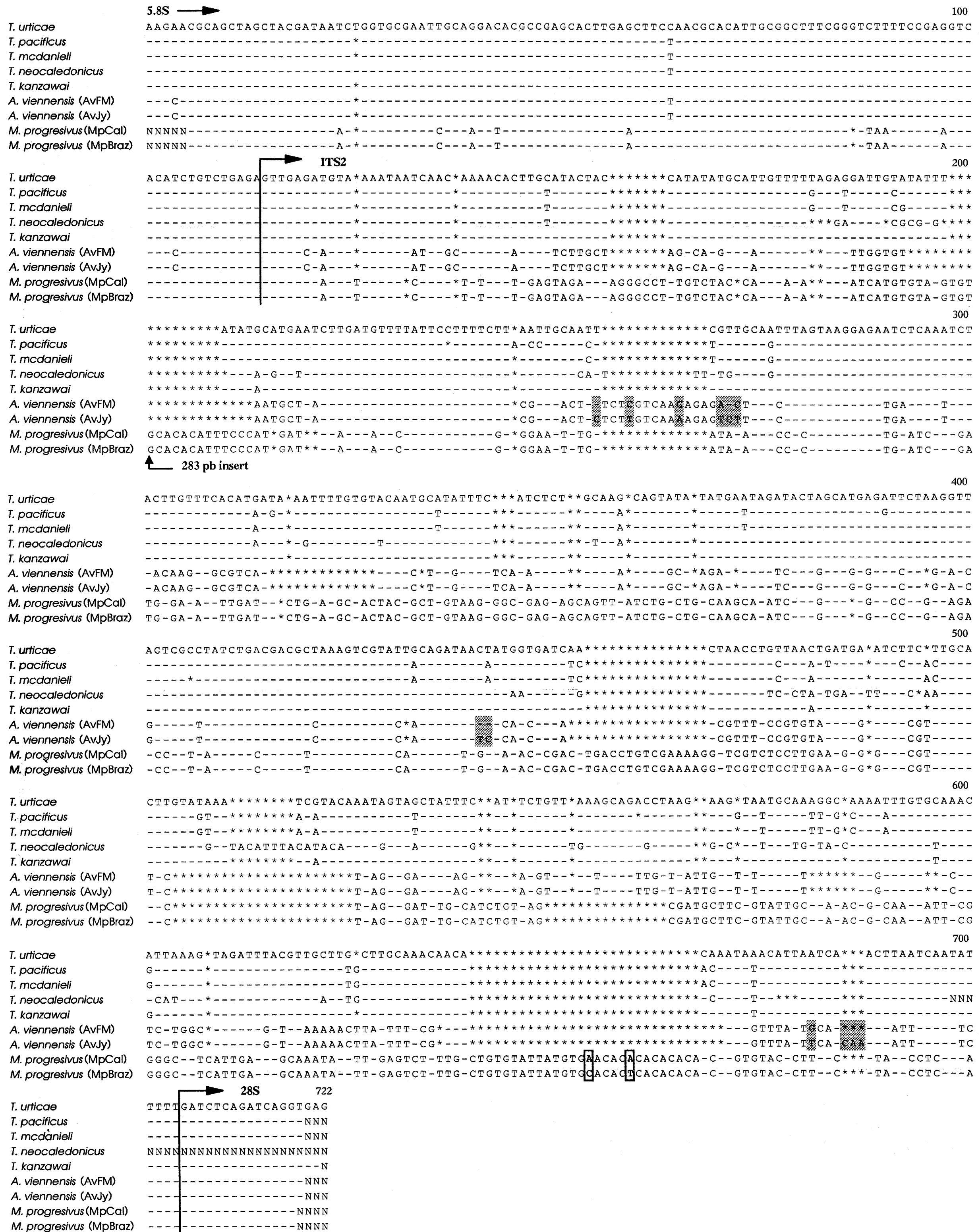

Fig. 2 Partial 5.8S and complete ITS2 sequences (5′ to 3′) of *Tetranychus urticae* and six other tetranychid mite species. Identities with the *T. urticae* sequence are indicated by dashes, and asterisks represent gaps. A 283 bp insertion in position 201 was detected in the *Mononychellus progresivus* sequence. Intraspecific comparisons are provided for strains of *Amphitetranychus viennensis* and *Mononychellus progresivus* and polymorphic sites are highlighted.

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Table 2 Pairwise nucleotide distance (upper-right matrix) and standard errors (lower-left matrix) among five tetranychid mite species. The Tamura (1992) correction for multiple substitutions was used based on: (a) ribosomal ITS2 sequences and (b) mitochondrial *COI* sequences. Positions with a gap in one of the sequences were excluded

	T. urticae	*T. kanzawai*	*T. mcdanieli*	*T. pacificus*	*T. neocaledonicus*
(a)					
Tetranychus urticae		0.013	0.077	0.089	0.149
T. kanzawai	0.005		0.072	0.084	0.141
T. mcdanieli	0.013	0.013		0.015	0.148
T. pacificus	0.014	0.014	0.007		0.161
T. neocaledonicus	0.020	0.019	0.019	0.020	
(b)					
Tetranychus urticae		0.062	0.075	0.088	0.115
T. kanzawai	0.014		0.075	0.078	0.102
T. mcdanieli	0.016	0.016		0.055	0.108
T. pacificus	0.017	0.016	0.013		0.116
T. neocaledonicus	0.020	0.018	0.019	0.020	

Japan (Fig. 2). Four different localities were sampled in France and two in Japan and all the mites in each country had identical ITS2 sequences.

Mitochondrial COI *intraspecific variation*

The sequence of a *COI* fragment (390 bp) was determined in 24 *T. urticae* individuals also analysed for ITS2 (Table 1). Comparisons of *T. urticae* sequences revealed 26 variable positions, all of which involved only point mutations (Fig. 4). The analysis of variable sites shows that the *COI* sequences cluster in two major groups referred to as lineages I and II. Nine of the variable sites are diagnostic between these two lineages. Lineage I contains either red or green forms of mites originating only from the Mediterranean area and collected from *Phaseolus* sp. (FA), *Malva* sp. (TN) and *Citrus* spp. (GR, EO, EV). Lineage II, in addition to other Mediterranean origins (FM, IF, IP and ET) includes all other samples originating from four different continents. The phylogenetic tree inferred from the *COI* sequences is shown in Fig. 5. High bootstrap scores give confidence to support the existence of two major lineages. The nucleotide divergence between these lineages is about 5.0% on average. Branching orders inside each lineage are not well resolved by this short sequence and will not be discussed.

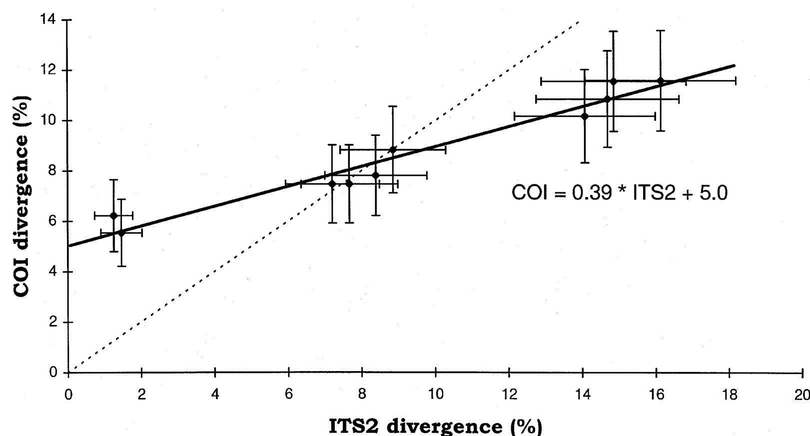

Fig. 3 Plot of *COI* vs. ITS2 divergences in pairwise comparisons of five *Tetranychus* species (Table 2). Standard errors around the data points are indicated. The dotted line is the diagonal (*COI* = ITS). The thick line is the linear regression, the equation of which is shown on the graph.

Species	Strain	37	90	101	105	115	150	168	180	192	195	198	201	207	210	213	240	246	250	267	276	312	324	330	345	375	381	
T. urticae	LR	G	G	T	A	T	T	T	T	T	T	A	T	T	C	T	T	A	T	C	T	T	T	A	A	T	T	
	R	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	
	NLs	.	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	C	
	NLw	.	.	.	.	.	A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	C	
	IF	.	.	.	.	.	A	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	C	Lineage II
	FM	.	.	.	.	.	A	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	A	.	.	.	C	
	J	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
	USA1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	?	.	.	.	.	
	ET	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	C	.	
	IP	.	.	.	.	.	A	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	A	.	.	C	.	
	FA	**A**	**A**	.	**T**	**C**	A	.	**C**	.	.	.	.	.	T	.	C	**T**	.	**T**	.	C	A	**G**	**T**	.	.	
	EV	**A**	**A**	.	**T**	**C**	A	C	**C**	.	.	G	.	.	T	A	C	**T**	.	**T**	C	C	A	**G**	**T**	C	C	
	EO	**A**	**A**	.	**T**	**C**	A	C	**C**	.	.	G	.	.	T	A	.	**T**	.	**T**	.	C	A	**G**	**T**	C	C	Lineage I
	GR	**A**	**A**	.	**T**	**C**	A	C	**C**	.	.	G	C	.	T	.	C	**T**	.	**T**	.	C	A	**G**	**C**	C	.	
	TN	**A**	**A**	.	**T**	**C**	A	C	**C**	.	.	G	.	.	T	.	C	**T**	C	**T**	.	.	A	**G**	**T**	C	.	

Species	Strain	135	150	204	210	216	240	246	283	301	324	345	363	381
A. viennensis	AvFL	T	T	T	T	A	T	T	A	C	G	T	C	T
	AvFV	.	.	.	.	.	.	.	.	.	.	.	.	.
	AvFM	.	.	.	.	.	.	.	.	.	.	.	T	.
	AvJy	A	A	C	C	G	C	A	T	T	A	A	T	C
	AvJa	A	A	C	C	G	C	A	T	T	A	A	T	C

Species	Strain	99	123	138	213	249	321	336
M. progresivus	MpBen	G	T	T	T	C	A	C
	MpCon	.	.	.	.	.	.	.
	MpCal	A	.	C	.	.	.	.
	MpBraz	A	C	C	A	T	G	T

Fig. 4 Variable nucleotide sites among *COI* sequences of samples of three tetranychid species. Abbreviations of collection sites are as in Table 1. The base numbers refer to the position in the published nucleotide sequence of *Tetranychus urticae* (Navajas *et al*., 1996). Dots indicate identity with the first sequence for each species. The nucleotide substitutions that are diagnostic between the two lineages of *T. urticae* are highlighted.

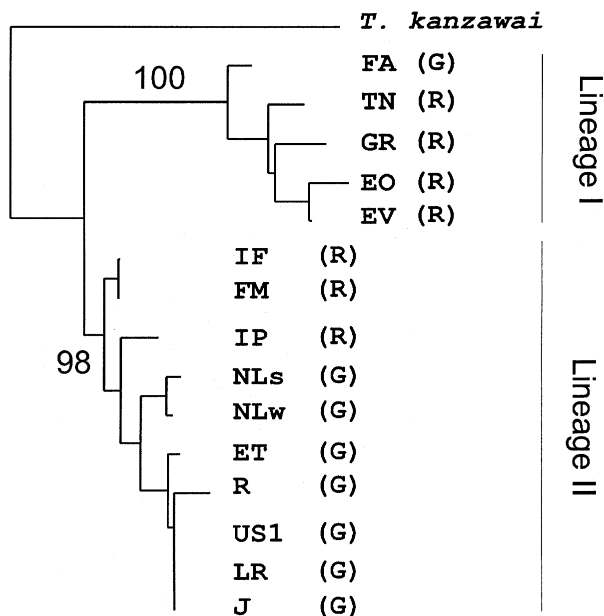

Fig. 5 Phylogenetic tree inferred from *COI* nucleotide sequences of various samples of *Tetranychus urticae*. The Neighbour Joining method was used based on distances calculated using Kimura's two-parameter correction method. The codes for each location are as in Table 1. The species *Tetranychus kanzawai* was used as outgroup. Bootstrap scores are indicated for the two major lineages. Mite colouration for each sample is indicated in brackets: (R) = red; (G) = green.

Intraspecific variation of *COI* sequences was also recorded in the two other tetranychid mites considered in this study, *A. viennensis* and *M. progresivus* (Fig. 4). Intraspecific differences in *COI* sequences in these two species are congruent with *ITS2* variation and correlate with the geographical origin of mite samples: 12–13 single nucleotide changes clearly differentiate the two French haplotypes of *A. viennensis* from the Japanese one (3.6–3.9% divergence). The *M. progresivus* mites from Brazil differ from all other origins by five to seven substitutions (1.5–2.1% divergence). Two sites are diagnostic between the American and the African samples. There were no intrasample differences in any of the species investigated.

Discussion

Our study shows complete homogeneity of ITS2 consensus sequences in different populations of *T. urticae* collected from several host plants in an extensive geographical area. In contrast, we found for the same mites substantial divergence in the *COI* sequences (around 5% between two major lineages that were detected). This contrast does not appear to be linked to a slower evolution rate of ITS2. In fact, the comparison of interspecific divergences of ITS2 and *COI* in Fig. 3 rather suggests that ITS2 evolves about 2.5 times faster than *COI* in the genus *Tetranychus*. The fact that the regression of *COI* against ITS2 divergences does not go through the

origin means that *COI* divergence predates species divergence to a greater extent than does ITS divergence. In fact, a retention of ancestral polymorphism may cause molecular divergence to be substantially older than species divergence as was for instance reported in two extreme examples involving closely related species of mice (Fort *et al*., 1984) and hares (Pérez-Suarez *et al*., 1994). Our results on mites could mean that ancestral polymorphism is generally higher for *COI* than for ITS2, but this is not the only possible explanation. As a result of the method used (direct sequencing of PCR products amplified from genomic DNA) we determined the consensus ITS sequences of the different species. This sequence is likely to be identical to, or close to, the ancestor of the sequences of the different copies of ITS2 in the genome of the species, and we are thus blind to intraspecific polymorphism between copies of the sequence. On the other hand, the polymorphism that was present in the ancestral species is not hidden, because it becomes observable once the descendants of alternative copies have been fixed in the genomes of the sister species. Thus species divergence times based on ITS consensus sequence divergence should be biased downwards as result of overlooking intraspecific polymorphism, but upwards because polymorphism of the ancestral species is incorporated in this divergence. Overall, ITS consensus sequence divergence should thus provide a more or less accurate estimate of species divergence time, provided levels of intraspecific polymorphism do not vary too much over time and between lineages. That the regression of *COI* against ITS2 divergence does not go through the origin (Fig. 3) is not necessarily accounted for by ancestral polymorphism being higher for mtDNA, but could also be explained by the fact that ancestral polymorphism is indirectly corrected for when measuring interspecific ITS consensus sequence divergences (note that these explanations are not exclusive). This would mean that ancestral polymorphism for *COI* is of the order of 5% (see the intercept of the regression in Fig. 3). This appears a plausible value, because we found an average of 5% divergence between the two major *COI* lineages in *T. urticae*, and 3.9% between haplotypes sampled in *A. viennensis.*

The reasoning above applies whatever the amount by which individual copies of ITS vary from the consensus sequence of the species. This amount depends on the efficiency of the concerted evolution process, which presumably results from a variety of mechanisms of nonreciprocal DNA transfer, including gene conversion (Nagylaki, 1984), unequal crossing-over (Smith, 1976; Dover, 1982), and intrachromosomal exchanges (Schlötterer *et al*., 1994). The efficiency of the concerted evolution process in *T. urticae* cannot be assessed from our data because we did not determine the sequence of individual repeats of the ITS2. However, the invariance of the ITS2 consensus sequence over the species range would seem to indicate a certain homogeneity of the rDNA pool of this species.

Our mtDNA polymorphism data in *T. urticae* allow us to judge the homogeneity of this species. It has long been considered as a complex of species because of differences in morphology, haemolymph pigments and response to photoperiod, and of results of cross-breeding experiments (Bund & Helle, 1960; Parr & Hussey, 1960; Boudreaux, 1963; Veerman, 1970, 1974). Partial incompatibilities between closely adjacent populations were found in agricultural as well as in natural environments (Helle & Pieterse, 1965; De Boer, 1985), and might be related to the presence of *Wolbachia* (Tsagkarakou *et al*., 1996), an endosymbiont which is involved in cytoplasmic incompatibilities (Barr, 1982) and may be associated with genetic and phenotypic variation. Studies of nuclear gene polymorphisms at a restricted geographical scale have revealed patterns of isolation by distance and sometimes by host plant (Tsagkarakou *et al*., 1997, 1998). The analysis of mtDNA variation reveals the existence of very divergent lineages, but no correlation between mtDNA divergence and either host plant or physiological characteristics, such as red *(T. urticae cinnabarinus*) or green haemolymph (*T. urticae sensu stricto*) (Table 1 and Fig. 5).

However, on the geographical scale sampled in this study and given the time spanned by the observed *COI* variation, the present mtDNA data reflect the colonization history of the species rather than local and labile differentiation. The Mediterranean region possesses the highest phyletic mtDNA diversity; in addition, one of the two lineages detected (lineage I) consists entirely of Mediterranean samples (Figs 4 and 5). Seen together, these two points appear to indicate substantial age of colonization in the region. It is noteworthy that a very little diversified clade includes most of the non-Mediterranean origins sampled: R (Russia), US1 (USA), LR (Latvia) and J (Japan), suggesting that non-Mediterranean regions of the northern hemisphere were recently colonized by a subset of the Mediterranean population. Inside the Mediterranean region, there is no apparent phylogeographical structure in the data, and thus no evidence of long-standing genetic isolation between geographical regions. This and the evidence for recent coloniza-

tion of the northern temperate regions fits well with the absence of rDNA differentiation. Furthermore, the fact that substantial mtDNA polymorphism and divergence (up to 5% between the major lineages) is found in this species indicates that a bottleneck is unlikely to be responsible for the homogenization of ITS sequences over the whole species range.

Species-wide homogenization of rDNA sequences does not appear to be a constant in mites; a certain variability in ITS2 sequences was detected in the two other mite species analysed for this purpose. In *A. viennensis*, we found 2.0% ITS2 divergence between the French and Japanese samples, which differed by 3.6–3.9% for *COI*. The divergences are more reduced in *M. progresivus*, which showed about 2% *COI* divergence, but only 0.25% ITS2 divergence, between the Brazilian samples and the others. In both cases however, different ITS2 sequences were only found between samples that also had the most divergent *COI* sequences, indicating a concordance of the information of the two markers, and suggesting the absence of gene flow between regions with different ITS2 sequences.

The results reported here may be related to ecological factors, with the feeding and ecological specificity of the mite species providing a key factor conditioning the opportunities of long-distance migration and colonization. In contrast to the highly polyphagous *T. urticae*, *M. progresivus* is a monophagous tetranychid that feeds only on cassava plants, and *A. viennensis* is oligophagous and feeds only on Rosaceae. Furthermore, the colonization abilities of these last two species appear to be limited by factors other than host plant availability. The American species *M. progresivus* follows the distribution of the host plant, *Manihot esculenta*, in Central and South America. However, the mite has established only in Africa, in spite of the presence of cultivated cassava in other continents (south-east Asia, USA). Similarly, the distribution of *A. viennensis* is restricted to Eurasia, despite the recurrent introductions of cultivated Rosaceae worldwide.

A better knowledge of the mechanisms and kinetics of concerted evolution of rDNA is clearly needed before the relative performances of rDNA and mtDNA in detecting historical barriers to gene flow can be fully assessed. It is not known whether the homogenization of rDNA sequences in an isolated population takes more or less time than the sorting of mtDNA lineages leading to the monophyly of the population for this marker. In any case, the conjunction of information on different markers is preferable to the reliance on the particular history of a single gene.

Acknowledgements

We are grateful to C. Schlötterer for helpful comments on the manuscript. We thank B. Michot for performing secondary structure analysis of *ITS2* sequences. We also thank the following for kindly providing mite specimens: H. Bolland, University of Amsterdam, The Netherlands; O. Bonato, ORSTOM, Montpellier, France; G. Fédière, ORSTOM, Cairo, Egypt; F. Garcia-Mari, Universidad Politecnica Valencia, Spain; G. Fauvel, INRA, Montpellier, France; T. Gotoh, Ibaraki University, Japan; R. Karban, UCDavis, USA; S. Kreiter, ENSAM, Montpellier, France; A. Tsagkarakou, Benaki Institute, Greece; H. Thistlewood, Agri-Canada, Vineland, Canada; and D. Walsh, UCDavis, USA. This is contribution ISEM96 104.

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