Intraspecific diversity of the Cassava Green Mite *Mononychellus progresivus* (Acari: Tetranychidae) using comparisons of mitochondrial and nuclear ribosomal DNA sequences and cross-breeding

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ABSTRACT

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Intraspecific diversity in Cassava Green Mite *Mononychellus progresivus* Doreste was examined using individuals collected in Benin and the Congo and in Columbia and Brazil. Comparisons were based on mitochondrial and ribosomal DNA sequences and the results of several cross-breeding experiments. Sequence variation was examined in a total of 1139 base pairs (bp) constituting the ITS2 ribosomal DNA (805 bp) and a fragment of the Cytochrome Oxidase I (COI) gene (334 bp). Sequence divergence is low, ranging from 0% to 2.1% for COI and from 0% to 0.4% for ITS2. Inter-strain comparisons have shown that the two African populations appear to be identical. They were similar to the Colombian population while the Brazilian population was clearly different. The data support the hypothesis of a single introduction of the species in the two African populations. Crossing experiments have shown partial hybrid sterility, suggesting a genetic incompatibility consistent with differences detected by sequence data. The results show the usefulness of molecular markers as a tool for determining taxonomic status and dispersion paths in spider mites.

INTRODUCTION

There are many examples of intraspecific diversity in Tetranychidae; these are shown by differences in both morphological characteristics (Boudreaux, 1956, 1963;

Gutierrez, 1987) and behaviour with regard to an environmental factor (de Boer, 1985; Veerman, 1985; Gotoh *et al.*, 1993) or in enzymatic systems (Ward *et al.*, 1982; Sula and Weyda, 1983; Gotoh and Takayama, 1992; Osakabe and Sakagami, 1993; Osakabe *et al.*, 1993).

The genetic basis of phenotype variability observed is not known, but reproductive incompatibility between populations reported in most of the taxa studied is proof of genetic divergence between isolates of a species. Those concerned in particular are several representatives of the genus *Tetranychus* (Helle and Pieterse, 1965; Gutierrez and van Zon, 1973; Overmeer and van Zon, 1976; de Boer, 1985; Fry, 1989; Gotoh and Takayama, 1992; Gotoh et al., 1993) and *Panonychus citri* (McGregor) (Inoue, 1972).

Haplo-diploid sex determination (the most common reproduction system in Tetranychidae species) has particular implications with respect to the maintenance of genetic diversity. It has been suggested that interpopulation variability is enhanced by haplodiploidy while the intrapopulation variability is reduced (Helle and Overmeer, 1973).

Mononychellus progresivus Doreste, which originated in the neotropical region and feeds mainly on cassava, was introduced accidentally to East Africa (Uganda) in the early 1970s (Lyon, 1973). This first outbreak resulted in the infestation of most cassava plantations in Africa (Yaninek and Herren, 1988). Variability in the length of dorsal setae from one strain to another is so obvious that it was believed for several years that there were a number of distinct taxa. However, the analysis of stable morphological characters (chaetotaxy of legs and shape of aedeagus) in several samples from different sites in East Africa (Kenya), Central Africa (Congo and Central African Republic) and West Africa (Benin and Ivory Coast) tends to prove that the forms observed are identical (Gutierrez, 1987). A series of crosses undertaken in East Africa by Murega (1989) showed the compatibility of the six populations from Kenya and Uganda, with egg mortality of less than 10%. It was therefore considered of interest to compare these African populations which appear to not be genetically isolated, with South American populations. The African populations were introduced recently probably from a single source.

In recent years, evaluation of genetic variability using nucleotide sequences of DNA has made it easier to characterise species diversity and has made it possible to determine the status of the taxa studied. The Polymerase Chain Reaction method (PCR; Saiki *et al.*, 1988) has become one of the more powerful tools used in molecular biological studies. Using this technique, specific short regions of a gene can be greatly amplified *in vitro* from an extremely small amount of DNA.

In this study, PCR has been used to detect polymorphic DNA sequences from single mites. Variable mitochondrial and nuclear DNA sequences studied include: (i) a fragment of the mitochondrial cytochrome oxidase I (COI) gene and (ii) one of the internal transcribed spacer (ITS 2) of the ribosomal RNA transcript. Hillis and Dixon (1991) and Wolstenholme (1992) respectively have published general

descriptions of mitochondrial and nuclear genomic regions. Their utility as genetic markers of diversity has been shown for several groups of organisms. In mites, the internal transcribed spacers have been used to investigate the validity of species status in the genus *Ixodes* (Acari: Ixodidae) (Wesson *et al.*, 1993). Moreover, a preliminary characterisation of ITS2 sequences in Tetranychidae displayed a divergence rate that is useful in the comparison of closely related taxa in this family (Navajas *et al.*, 1992). On the other hand, the COI gene has been characterised in *Tetranychus urticae* Koch (Fournier *et al.*, 1994) and also used to investigate diversity in Tetranychidae (Navajas, *et al.*, 1994).

A comparison of COI and ITS2 nucleotide sequences of individual mites belonging to four distinct populations of *M. progresivus* collected on cassava are described here. The mites examined were from two distinct populations from distant parts of Africa (the Congo and Benin) and from two other geographically separated regions in South America (Colombia and Brazil). The degree of genetic isolation of the populations was also assessed by compatibility in a series of intra and inter-population crosses.

MATERIALS AND METHODS

Mites from the Congo (CG) and Benin (BN) were originally collected in the field from Brazzaville and Cotonou respectively and then maintained as laboratory colonies. Mites from Colombia (CL) and Brazil (BR) were from long-established stocks originally collected in Cali and Petrolina (Pernambuco – Nordeste).

PCR-amplification and sequencing

DNA extraction was performed using single fresh individuals. Mites were homogenized in a microtube in 40 µl of 5% (wt/vol) Chelex 100 chelating resin. The sample was incubated at 56°C for 30 min, vortexed at high speed for 15 sec and incubated at 95°C for 15 min. The tube was centrifuged again for 5 min to pellet the resin and 2 µl of the aqueous phase containing the DNA was used as template in PCR reactions.

The PCR was performed in a 50 µl volume reaction containing 2.5 units of *Taq* polymerase (Promega), 0.2 mM of dNTP and 1.4 µM of each of the two oligonucleotide primers. Amplification was carried out by an initial denaturation step of 95°C for 4 min followed by 35 cycles of denaturation at 92°C for 1 min, annealing at 50°C or 52°C for 1 min (for COI and ITS2 respectively) and extension at 72°C for 1 min. Amplification products were examined by electrophoresis on 0.8% agarose gels staining with ethidium bromide. Excess primers and nucleotides were removed from PCR products with Geneclean (Bio 101) according to the manufacturer's instructions, and one third of the eluted DNA was used for sequencing. Double-stranded amplification products were directly sequenced by the dideoxy chain-termination method (Sanger *et al.*, 1977), using the Sequenase 2.0 kit (US Biochemical Corp.) and α-^{35}S-dATP (Amersham) for labelling. After

electrophoresis, gels were fixed in 10% acetic acid and 10% ethanol, dried and autoradiographed. The amplification and sequencing primers used in this study are listed in Table 1.

Sequences were read and entered into computer files by a digitizing system (DNA Parrot DP 100-PC 2.1, Clontech). The alignments of sequences were unambiguous and assessed by eye.

Cross-breeding procedure

As no difference was observed between the African specimens in the sequences in question, crosses were only performed on material from three sources: CG, CL and BR.

Rearing was performed using several tens of individuals from each geographical source. Rearing and crossing operations were performed on cassava leaf discs 2.5 cm in diameter on moist cotton wool in an air-conditioned chamber set at 26 ± 0,5°C, relative humidity 70 ± 5% and subjected to a photoperiod of 12 h light (intensity 4,000 lux) and 12 h darkness.

In parallel with a control rearing operation with three pure populations, each of the latter was crossed with the two others in both directions, making a total of nine rearing or crossing operations.

In order to obtain each F_1, 31 females collected at the teliochrysalis stage were each reared separately on a leaf disc with two males of the population used for the cross. The eggs laid were counted each day to obtain the total laid by each female. The parents were moved to a new disc every five days until death of the females; the males were replaced if they died in the first ten days. The eggs were collected and the resulting larvae reared to adult stage to determine the hatching rate and the proportion of males and females in the F_1 stage.

Statistical analysis: the results were compared using analysis of variance

TABLE 1

Primers used in polymerase chain reaction amplification and sequencing of the ribosomal (ITS2) and mitochondrial (COI) DNA regions studied.

Name of primer	Sequence (5' to 3')	Position	Amplified region	Purpose of primer
LC1[1]	CGAGTATCGATGAAGAACGCAGC	5.8S	ITS2	PCR/Sequencing
HC2[1]	ATATGCTTAAGTTCAGCGGG	28S	ITS2	PCR/Sequencing
A21[2]	CGACTTTAGCGTCGTCAGAT	ITS2	ITS2	Sequencing
668[2]	TGCAGGACACGCCGAGCACT	ITS2	ITS2	Sequencing
133[2]	TATCTAGATCATGGGAAAT	ITS2	ITS2	Sequencing
772[2]	TGATTTTTTGGTCACCCAGAAG	COI	COI	PCR/Sequencing
773[2]	TACAGCTCCTATAGATAAAAC	COI	COI	PCR/Sequencing

[1]Primer described in Navajas *et al.* (1992).

[2]Primer designed in this study.

followed by Scheffe's F test (Scheffe, 1959) with 5% risk.

RESULTS

Nucleotide sequences

The full ITS2 (805 bp) and a fragment of the coding COI gene (334 bp) were amplified by PCR and sequenced for the individuals of the four populations of *M. progresivus* concerned. The sequences for the two genomic regions are shown in Figure 1 for the specimens from the Congo. Sequence alignment was un-

a)

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AGAAGTATAAATACCAACTATACATTGAGTAGATACAGGGCCTCTGTCTACCATGTATAG   60
AGATCATGTGTATGTGTGTATATCTGTGCATGGTATAACACATACTATGTGTACATGTAT  120
GTATCTTGCAGGATCGTAGCGCTTTAACTGGAAACGGTTAAGGTTAGCTACACGCCTGCA  180
TATAATTTACAGAAAGTATGTACCTTGCCTATGACTGGAAACAGTTATATCGTAATCTAC  240
ATATTAGTATGGATATTATATTGTTCCTTGCGTGTTATGTGGTAAAACACATATCATTGC  300
CGGTCAGAGATATATATGTATGTTACATAGTATGGTTTATCAATTTTTGTACTTAGTTTT  360
ACACACATTTCCCATGATCTAGATATTCTATTCCTTTTCGTGGAAGTATGTATATACAAC  420
CTCGTAAGGAGATGCATCAATGATGTGATATCTTGATATCTGTATGCTACTACAGCTAGT  480
AAGCGGCAGAGCAGCAGTTGATCTGACTGTCAAGCAAATCACTGGCAGGGACCCTGAGAG  540
AACCCGTCAATCTGCCGACGTTAAAGTCGTACAGCAGATTAGTAAGACGCGACATGACCT  600
GTCGAAAAGGTTCGTCTCCTTGAAAGGGGTCTCGTTTGCACTCTAAGGTGATTTGTCATC  660
TGTTAGCGATGCTTCTGTATTGCAGACACAGACAAGTATTACGGGGCAATCATTGATTAG  720
CAAATATGTTTGAGTCTCTTGACTGTGTATTATGTGAACACACACACACACCAAGTGTAC  780
TCTTTCCATATACCTCATAATTTTG
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b)

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TTAGTTATAATTTAGGTAAAAAAGAAGTATTTGGTAAATTAGGAATAATATTTGCTATGA   60
TATCAATTGGTTTATTAGGATTTGTTGTATGAGCTCATCATATATTTACTATTGGTATAG  120
ATGTTGATACTCGAGCTTATTTTACAGCAGCTACAATAATTATTGCTATCCCTACAGGTA  180
TTAAAATTTTTAGATGATTTACTACTATCATTAATTCTCATATTAATTTTAATATTTCTG  240
TCTATTGATCAATAGGATTTTTAATTATATTTTCTATTGGAGGTTTTACAGGAATCATTG  300
CTTCAAATTCTTGTTTAGATATCTCTTTACATGA
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Fig. 1. Sequences 5' to 3' of *Mononychellus progresivus* for (a) the full ITS2 ribosomal DNA and (b) a fragment of mitochondrial COI gene. The sequences shown are for individuals collected in the Congo. Variable positions among the 4 origins analysed are shown by asterisks.

ambiguous and the variable positions detected among the 1,139 nucleotides sequenced are shown in Table 2. ITS2 alignments include only one insertion/deletion (4 bp) at terminal 3' of the Brazil sequence and two single mutations in the same sequence. The three other sequences analysed are identical. In addition, seven positions are variable in the COI sequenced region. The sequences of the different individuals of the four strains were compared two by two (Table 3). Analysis showed that two mutations separated American and African sequences (positions 59 and 98 in COI) into two distinct groups, whereas five mutations characterised the sequence (COI) from Brazil. Sequences of ITS2 and COI from Congo and Benin were identical (tables 2 and 3).

No intra-strain variability was detected. This was verified for the sequences of four individuals from the Congo; three from Colombia, two from Benin and two from Brazil.

Furthermore sequences have been aligned to those of other Tetranychidae: 19 species for COI and 10 species for ITS2 (Navajas *et al.*, 1994). In the case of ITS2,

TABLE 2

Nine variable positions among the 1,139 nucleotides sequenced (Fig. 1). The nucleotide at each variable position in the Congo sequence is shown. In the other types, the nucleotide is shown when different from the Congo, with identity indicated by a dot. In addition, the Brazil sequence has a four-nucleotide insertion in the ITS2, not found in any of the other individuals studied.

	COI							ITS2	
	59	83	98	173	209	281	296	757	762
Congo (CG)	G	T	T	T	C	A	C	T	T
Benin (BN)	.	.	.	.	.	.	.	.	.
Colombia (CL)	A	.	C	.	.	.	.	.	.
Brazil (BR)	A	C	C	A	T	G	T	G	A

TABLE 3

Pairwise sequence differences among the four populations of *Mononychellus progresivus* studied. The figures show the number of nucleotide differences in ribosomal ITS2 (above the diagonal) and mitochondrial COI (below).

		CG	BN	CL	BR
CONGO	(CG)	–	0	0	3
BENIN	(BN)	0	–	0	3
COLOMBIA	(CL)	2	2	–	3
BRAZIL	(BR)	7	7	5	–

the alignments were adjusted with studies of the secondary structure. The alignment analysis agreed with classical systematics.

Cross-breeding

In each rearing or crossing operation, one, two or three of the initial females were lost by drowning or disappeared without laying. To enable statistical analysis of the results, all the calculations were performed on the basis of 28 females, with elimination, when necessary, of female/s which had laid the least.

A matrix of the rearing and crossing of the taxa and the average number of eggs per female in each one is provided in Table 4. Column F1 shows the number of females and males obtained and the number of unhatched eggs. Laying was extremely homogeneous except in cross BR × CG where it was significantly more substantial. Such differences in total fecundity have been observed in crossings between other tetranychid populations (Gutierrez and van Zon; 1973) without

TABLE 4

Results of rearing and crosses between the four populations of *Mononychellus progresivus* studied. Data are means ± SEM. Distribution in percentage of F_1 is indicated in brackets. Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$) (Scheffe's F test).

Parents		F_1		
Females × males	Mean number of eggs per female	females	Mean number of males	non-viable eggs
CG × CG	25.9±4.0a	18.2±3.1ab *(70%)*	6.6±1.2a *(26%)*	1.1±0.4a *(4%)*
CG × CL	19.8±2.5a	13.2±2.1ab *(67%)*	4.9±0.9a *(25%)*	1.7±0.4ab *(8%)*
CG × BR	32.2±7.5a	11.2±3.4a *(35%)*	7.9±1.7a *(24%)*	13.1±5.0cef *(41%)*
CL × CL	25.0±4.1a	18.9±3.2b *(76%)*	5.1±0.8a *(20%)*	1.0±0.4a *(4%)*
CL × CG	24.2±4.2a	11.4±3.0ab *(47%)*	7.6±1.9a *(31%)*	5.2±1.9bdeg *(22%)*
CL × BR	26.0±5.4a	13.0±3.5ab *(50%)*	5.9±1.9a *(23%)*	7.1±2.2cdeg *(27%)*
BR × BR	29.6±4.0a	19.1±3.2ab *(64%)*	9.4±1.2ac *(32%)*	1.1±0.4a *(4%)*
BR × CG	52.2±7.5b	3.9±1.4c *(8%)*	31.0±5.9b *(59%)*	17.3±2.5f *(33%)*
BR × CL	38.6±8.3a	16.0±3.4ab *(42%)*	19.4±5.3bc *(50%)*	3.2±1.0adeg *(8%)*

precise explanation.

These mites exhibit arrhenotokous parthenogenic reproduction; males hatch from unfertilised haploid eggs and females hatch from diploid eggs. Here, all the populations are genetically compatible since females were obtained from all the crosses tested.

The cross direction affected the number of females and males produced when individuals from CG and BR were used. The sex ratio in the BR x CG cross was strongly affected, with a very small number of females ($p<0.05$). In addition, whereas the number of unviable eggs was a little higher than in the pure populations in CL x CG alone (22%) or in CL x BR alone (27%) in crosses of these populations, it was significantly higher in the two crosses CG × BR (41%) and BR × CG (33%), corresponding to greater incompatibility between the two latter populations.

DISCUSSION

The crossing data, as a whole, show that the four strains studied are genetically compatible, indicating that the *Mononychellus* populations studied are conspecific. These results underline the interest of the two principal criteria retained for identification of these Tetranychidae at the species level: shape of aedeagus and leg chetotaxy .

These results are supported by the low number of nucleotide differences. Nucleotide divergence varied from 0 to 2.1% for COI and from 0 to 0.4 % for l'ITS2. These figures are lower than those obtained in an other study for the same DNA fragments for two very close but distinct species such as *T. urticae* and *T. turkestani* Ugarov and Nikolskii. Divergence between these species was 5 % and 1.9 % respectively. Divergence reaches 11 % (COI) and 22% (ITS2) when comparison covers species such as *T. urticae* and *M. progresivus*, which belong to different genera (Navajas, *et al.* 1994).

The sequences of the two DNA fragments analysed are identical for the African individuals. This suggests either that the two groups shared a common ancestor in the recent past or that they are bound by gene flow. The latter hypothesis seems less plausible because of the geographical distance between Benin and Congo. The data thus tend to confirm recent common origin of the two populations.

However, the fecundity data reveal divergence between the individuals from Colombia and Brazil, which may be explained by the interruption of gene exchange between the two populations. This partial hybrid sterility suggests a maternal effect already reported by several authors mentioned by de Boer (1985) and also reported recently to occur in *T. urticae* (Fry, 1989).

In addition, the two approaches lead to considering that the African populations are more closely related to the Colombian population than the Brazilian population. Introduction of *M. progresivus* to Africa thus probably occurred from a population closer to Colombia than to Brazil.

In order to refine these preliminary conclusions, it would of course be interesting to obtain a more complete picture of intraspecific diversity in *M. progresivus*. Assessment of the diversity of several American populations would make it possible to clarify the precise origin of the introduction/s of *M. progresivus* in Africa. Although recent introduction in Africa of *M. progresivus* from American stock has nevertheless been suspected, this is the first molecular evidence of the phenomenon.

Infra-specific determination in Tetranychidae may provide important information for the study of cosmopolitan pests which are frequently carried by man. Analysis of molecular genome markers may become an important tool to answer questions concerning the dispersion, genetical structuration of the species and the systematics of these mites.

Sequences availability

Sequences have been deposited in EMBL under accession numbers X79901 and X79902.

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REFERENCES

Boer, R. de, 1985. Reproductive barriers. In: W. Helle and M.W. Sabelis (Editors), Spider Mites, their Biology, Natural Enemies and Control. World crop Pests, Vol. IA, Elsevier, Amsterdam, pp. 193–199.

Boudreaux, H.B., 1956. Revision of the two-spotted spider mite complex. Ann. Entomol. Soc. Am., 49: 43–48.

Boudreaux, H.B., 1963. Biological aspects of some phytophagous mites. Annu. Rev. Entomol., 8: 137–154.

Fournier, D., Bride, J.M. and Navajas, M. 1994. Mitochondrial DNA from spider mites: isolation, restriction map and partial sequence of the Cytochrome Oxidase Subunite I Gene. Genetica (in press).

Fry, J.D., 1989. Nuclear-nuclear and nuclear-cytoplasmic interactions contribute to the reproductive incompatibility between two strains of the twospotted spider mite. Entomol. Exp. Appl., 50: 97–100.

Gotoh, T. and Takayama, K., 1992. Developmental characteristics, Genetic compatibility and esterase zymograms in three strains of the Hawthorn Spider Mite, *Tetranychus viennensis* Zacher (Acari: Tetranychidae). J. Acarol. Soc. Jpn., 1: 45–60.

Gotoh, T., Bruin, J., Sabelis, M.W. and Menken S.B.J., 1993. Host race formation in *Tetranychus urticae*: genetic differentiation, host plant preference, and mate choice in a tomato and a cucumber strain. Entomol. Exp. Appl., 68: 171–178.

Gutierrez, J., 1987. The Cassava Green Mite in Africa: one or two species? (Acari: Tetranychidae). Exp. Appl. Acarol., 3: 163–168.

Gutierrez, J. and van Zon, A.Q., 1973. A comparative study of several strains of the *Tetranychus neocaledonicus* complex and sterilization of males by X-rays. Entomol. Exp. Appl., 16: 123–134.

Helle, W. and Overmeer, W.P.J., 1973. Variability in tetranychid mites. Annu. Rev. Entomol., 18: 97–120.

Helle, W. and Pieterse, A.H., 1965. Genetic affinities between adjacent populations of spider mites (*Tetranychus urticae* Koch). Entomol. Exp. Appl., 8: 305–308.

Hillis, D.M. and Dixon, M.T., 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol., 66: 411–453.

Inoue, K., 1972. Sterilities, visible mutations in F_1 hybrid females obtained by crosses between different strains and mortalities of their eggs in Citrus red mites, *Panonychus citri* (McG.). Bull. Hortic. Res. Stn., 7: 29–36.

Lyon, W.F., 1973. A plant feeding mite *Mononychellus tanajoa* (Bondar) new to the African continent threatens cassava (*Manihot esculenta* Crantz) in Uganda. PANS, 19: 36–37.

Murega, T.N., 1989. Cross-breeding studies on the Cassava Green Spider Mite *Mononychellus* sp. (Acari: Tetranychidae) in East Africa. Exp. Appl. Acarol., 6: 85–90.

Navajas, M., Cotton, D., Kreiter, S. and Gutierrez, J., 1992. Molecular approach in spider mites (Acari: Tetranychidae): preliminary data on ribosomal DNA sequences. Exp. Appl. Acarol., 15: 211–218.

Navajas, M., Fournier, D., Gutierrez, J., Lagnel, J. and Boursot, P. 1994. Evolution of mitochondrial cytochrome oxidase subunit I sequences, morphological and life history traits in Tetranychidae mites. Mol. Biol. Evol. (submitted).

Osakabe, M. and Sakagami, Y., 1993. Estimation of genetic variation in Japanese populations of the Citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae) on the basis of esterase allele frequencies. Exp. Appl. Acarol., 17: 749–755.

Osakabe, M., Saito, Y. and Sakagami, Y., 1993. Protein differences detected by two dimensional electrophoresis in the species complex of *Schizotetranychus celarius* (Banks). Exp. Appl. Acarol., 17: 757–764.

Overmeer, W.P.J. and van Zon, A.Q., 1976. Partial reproductive incompatibility between populations of spider mites. Entomol. Exp. Appl., 20: 225–236.

Saiki, R., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239: 487–491.

Sanger, F., Nicklen, S. and Coulson, A.R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci., 74: 5463–5467.

Scheffe, H., 1959. The analysis of variance. J. Wiley, New York, 477 pp.

Sula, J. and Weyda, F., 1983. Esterase polymorphism in several populations of the two-spotted spider mite, *Tetranychus urticae* Koch. Experientia, 39: 78–79.

Veerman, A., 1985. Diapause. In: W. Helle and M.W. Sabelis (Editors), Spider Mites, their Biology, Natural Enemies and Control. World Crop Pests, Vol IA, Elsevier, Amsterdam, pp. 279–316.

Ward, P.S., Boussy, I.A. and Swincer, D.E., 1982. Electrophoretic detection of enzyme polymorphism and differentiation in three species of spider mites *(Tetranychus)* (Acari: Tetranychidae). Entomol. Soc. Am., 75: 595–598.

Wesson, D.M., McLain, D.K., Oliver, J.H., Piesman, J. and Collins, F.H., 1993. Investigation of the validity of species states of *Ixodes dammini* (Acari: Ixodidae) using rDNA. Proc. Natl. Acad. Sci. USA, 90: 10221–10225.

Wolstenholme, D.R., 1992. Animal mitochondrial DNA: structure and evolution. Int. Rev. Cytol., 141: 173–216.

Yaninek, J.S. and Herren, H.R., 1988. Introduction and spread of the Cassava Green Mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), an exotic pest in Africa and the search for appropriate control methods: a review. Bull. Entomol. Res., 78: 1–13.