Bulletin of Entomological Research (2001) **91**, 117–123 DOI: 10.1079/BER200073

Synonymy between two spider mite species, *Tetranychus kanzawai* and *T. hydrangeae* (Acari: Tetranychidae), shown by ribosomal ITS2 sequences and cross-breeding experiments

M. Navajas[1]*, J. Gutierrez[1], M. Williams[2] and T. Gotoh[3]

[1]INRA, Centre de Biologie et Gestion de Populations, Campus International de Baillarguet, CS 3001 Montferrier-sur-Lez, 34988 Saint Gely du Fesc, France: [2]Department of Primary Industries, Water and Environment, 13 St Johns Avenue, New Town 7008, Tasmania, Australia: [3]Laboratory of Applied Entomology and Zoology, Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300–0393, Japan

Abstract

Amplification of the second internal transcribed spacer (ITS2) of ribosomal DNA was used to compare seven samples of the *Tetranychus kanzawai* Kishida–*T. hydrangeae* Pritchard & Baker mite complex from five different countries: Australia, the Congo, Indonesia, Japan and the USA. No morphological differences were detected between these mites and their ITS2 sequences displayed strong similarity except for a small nucleotide divergence of 0.2% in specimens from Australia and Indonesia. Reciprocal crosses and backcrosses between mites assumed to be *T. kanzawai* and *T. hydrangeae* respectively showed reproductive compatibility. Fertile hybrid females were obtained in all cases, indicating conspecificity of the mites tested. It is concluded that *T. hydrangeae* is a synonym of *T. kanzawai*. The evidence suggests that *T. kanzawai* originated in South-east Asia and probably spread throughout the world on *Hydrangea* spp. cuttings.

Introduction

The identification of spider mites, Tetranychidae, is not always easy for non-specialists because of the small size of these organisms. Distinction between species is often based only on a limited number of morphological characters. For example, the lobes of the dorsal hysterosomal striae of the body and the chaetotaxy of legs are often similar in species of the genus *Tetranychus* close to *Tetranychus urticae* Koch. The mediodorsal spur of empodia is not always obvious and numerous systematics specialists use the shape of the aedeagus, although the latter displays small individual variations and its size depends on mounting techniques. A certain amount of confusion has thus emerged concerning the taxon *Tetranychus hydrangeae* Pritchard & Baker, that some systematics specialists consider valid (Ehara & Wongsiri, 1975; Meyer, 1987; Baker & Tuttle, 1994) whereas others consider that it is synonymous with *Tetranychus kanzawai* Kishida, (Wainstein, 1960; Gutierrez & Schicha, 1983; Schicha & Gutierrez, 1985; Bolland *et al.*, 1998). The complexity of the problem is accentuated by the fact that *T. kanzawai* is extremely polyphagous in many countries in South-east Asia, attacking cassava and tea plantations and orchards, whereas in the rest of the world, *T. hydrangeae* is generally considered as a minor species associated with *Hydrangea* spp. (Saxifragaceae). This apparent confusion has considerable economic consequences since the quarantine services of countries considered to be free of *T. kanzawai* have the right to refuse the importation of plant material and

E-mail: navajas@ensam.inra.fr

fruits from infested regions. The economical risk is increased by the arrhenotokous mode of reproduction of spider mites, because a single egg may start a strain.

All of the microscope preparations of samples of the 'complex' received or prepared from our own collecting (in Australia, the Congo, Indonesia, Japan, Papua New Guinea and Thailand) appeared to be morphologically identical, but, given the difficulties of simple observation, doubts remained. It was thought that molecular biological techniques together with cross-breeding experiments might provide new, definitive data on the question of the true taxonomic status of these mites. A comparison was therefore undertaken of the nucleotide sequences of the second internal transcribed spacer (ITS2) of ribosomal DNA (rDNA) in samples from Japan assumed to be *T. kanzawai* and a sample collected in California assumed to be *T. hydrangeae*, together with morphologically identical samples from Australia, the Congo and Indonesia. One of the most popular markers used to infer phylogenetic relationships is ribosomal DNA. In eukaryotic organisms rDNA consists of three genes encoding the 18S, 5.8S and 28S subunits. Between these genes are the internal transcribed spacers 1 (ITS1, between the 18S and 5.8S gene) and 2 (ITS2, between the 5.8S and 28S gene) (Hillis & Dixon, 1991). The internal spacers evolve rather rapidly and are thus often useful in differentiating closely related taxa. Polymorphism within the internal spacers has been exploited to differentiate closely related organisms (Paskewitz *et al.*, 1993; Fenton *et al.*, 1995; Navajas *et al.*, 1997) down to the intraspecific level (Fritz *et al.*, 1994; Marinucci *et al.*, 1999; Navajas *et al.*, 1999) and has been successfully utilized in species diagnostic assays (Gotoh *et al.*, 1998). Genetic markers including sequences of ribosomal ITS2 have also been widely used to examine the existence of host-adapted races in phytophagous arthropods (Berlocher, 1999; Navajas *et al.*, 2000; Shufran *et al.*, 2000). Thus in the case of *T. hydrangeae*, which has been reported from hydrangea plants exclusively, the use of ITS2 data might elucidate whether it is a host race of *T. kanzawai*.

Cross-breeding experiments were undertaken to provide independent evidence of the taxonomic status of individuals within the *T. kanzawai–T. hydrangeae* complex. The reproductive compatibility between mites assumed to be *T. kanzawai* (collected on tea) and *T. hydrangeae* (collected on hydrangea plants) was checked by performing reciprocal crosses and backcrosses in the laboratory.

Brief historical background

Tetranychus kanzawai was described in 1927 by Kishida from specimens collected on mulberry *Morus* sp. (Moraceae) in Japan and redescribed by Ehara (1956), who also found it in Japan on several plants such as tea *Camellia sinensis* (Theaceae), hop *Humulus* sp. (Cannabaceae), apple tree *Malus domestica* (Rosaceae), plum tree *Prunus persica* (Rosaceae), pear tree *Pyrus communis* (Rosaceae), robinia *Robinia pseudoacacia* (Fabaceae) and vine *Vitis* sp. (Vitaceae). In the same publication, Ehara reported that *T. kanzawai* Kishida was synonymous with *T. japonicus* Hotta.

Pritchard & Baker (1955) described *T. hydrangeae* on the basis of mites collected on hydrangea *Hydrangea macrophylla* (Saxifragaceae), *Maranta* sp. (Marantaceae), beans *Phaseolus* spp. (Fabaceae) and *Euphorbia pulcherrima* (Euphorbiaceae) in California. In 1960, Wainstein considered that *T. hydrangeae* Pritchard & Baker, and *T. merganster* Boudreaux were synonymous with *T. kanzawai* Kishida. No other systematist has confirmed synonymy with *T. merganster*. In contrast, that of *T. hydrangeae* and *T. kanzawai* was proposed by Meyer (1974) for material collected from *H. macrophylla* in South Africa, and then by Gutierrez & Schicha (1983) for material collected in New South Wales and by Schicha & Gutierrez (1985) for material collected in Papua New Guinea.

In 1975, Ehara & Wongsiri reported that although the aedeagus of *T. hydrangeae* was very similar to that of *T. kanzawai*, it had a larger knob and its anterior projection was rounded and more conspicuous. According to these authors, the specimens collected in Thailand and those previously identified from Hong Kong on maize *Zea mays* (Poaceae) (Ehara & Lee, 1971) were probably *T. hydrangeae*. Following this article, Meyer (1987) changed her opinion, and proposed that the *Tetranychus* collected on *Hydrangea* in South Africa should be called *T. hydrangeae*. The remark by Ehara & Wongsiri (1975) was repeated by Ehara & Tho (1988) and then by Ehara & Masaki (1989), who confirmed the presence of *T. kanzawai* on numerous cultivated plants in Japan, Taiwan and Malaysia. However, Baker & Tuttle (1994) continued to use the name *T. hydrangeae* for the tetranychid mites collected on *Hydrangea macrophylla* in California and reported that the species was present in greenhouses in Wisconsin, USA. Lastly, Bolland *et al.* (1998) followed Wainstein's synonymy again and grouped all the collected material listed in the world literature under the names *T. kanzawai* or *T. hydrangeae*. This gave a list of 106 host plants and 19 infested countries or geographical entities.

Materials and methods

Biological material for DNA analysis

A list of the samples used for molecular analysis is provided in table 1. The morphology of all live material and specimens in 70% and 100% ethanol was examined and compared. Morphological features examined were the shape of the male aedeagus, the shape of the peritreme and the spinning eupathidium of the palpal tarsus and chaetotaxy of the body and legs. In the case of the sample from Japan collected on *Pyrus communis*, the morphological study could not be done because only one female in absolute alcohol was obtained, resulting from an interception by the Canadian Quarantine Service of a shipment of apples from Japan. The strain collected by P.A.V.D. Laan in Indonesia in 1977 was reared in the laboratory on *Phaseolus* sp. at Amsterdam University for several years and was examined in 1997.

It is interesting to note that *T. hydrangeae* was collected on *H. macrophylla* in Montpellier, France about 20 years ago by A. Rambier (G. Fauvel, personal communication) but we could not find it again, despite a thorough search being made.

DNA extraction, amplification and sequencing

The ribosomal ITS2 sequences were obtained for three specimens from each of the seven batches of collected material listed in table 1 (except for one of the samples from Japan). Field collections were preserved either frozen or in 100% ethanol until used. Total DNA was isolated from single adult females as follows. Mites were hydrated by immersing

each individual in 20 µl double distilled water three times for 10 s, 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 10 min. Proteins were removed with one volume chloroform/isoamyl alcohol (24:1) and DNA precipitated with one volume isopropanol. The resultant pellet was washed with 75% ethanol and resuspended in 20 µl double distilled water; 2 µl was used as the polymerase chain reaction (PCR) template.

The complete ITS2 region was PCR amplified with primers annealing to conserved regions of the 5.8S and 28S rDNA flanking subunits as detailed by Navajas *et al.* (1994). The same primers were used for sequencing. The PCR amplification and sequencing protocols as well as the primer sequences were as previously described for other tetranychid mites in Navajas *et al.* (1998). Intra-strain polymorphism was checked by analysing three individuals of each strain, except for the sample collected on apples from Japan where only an individual was studied. The sequences obtained were aligned by eye.

Crossing procedures

To determine whether gene flow could occur between individuals assumed to be either *T. hydrangeae* (Th) or *T. kanzawai* (Tk), crosses and backcrosses between these mites were performed. *Tetranychus hydrangeae* was also crossed with *T. parakanzawai* Ehara (Tp) a species closely related to *T. kanzawai* (Ehara, 1999).

Tetranychus kanzawai was collected at Kanaya (34°48'N, 138°23'E), Shizuoka, on May 19, 1993 from tea (*C. sinensis*) and *T. parakanzawai* was collected at Ami (36°01'N, 140°11'E), Ibaraki, on June 5, 1993 from Kudzu vine (*Pueraria lobata*, Fabaceae). *Tetranychus hydrangeae* collected from a hydrangea plant (*Hydrangea* sp.) in Sydney and kept in laboratory culture at Hobart, Tasmania, Australia, was imported to Japan (authorization no. 11 Yoko-534), on July 7, 1999. Each species was kept separately on leaf discs (*c.* 25 cm^2) of kidney bean and reared at 60–70% r.h., 25 ± 1°C and a 16L:8D photoperiod,

Females in the teleiochrysalis stage obtained from each stock culture were transferred onto a small leaf disc (*c.* 4 cm^2) with a male adult either from the same or a different culture. Males were removed two days after adult emergence of the females. Each female was allowed to lay eggs for five days and then removed. Eggs on leaf discs were checked each day to determine the hatchability, survival rate and sex ratio (% females).

Results

Morphology

No morphological differences were detected between the samples received and examined in the past (Australia, the Congo, Indonesia, Japan, Papua New Guinea and Thailand) or collected for this study (Australia, California, the Congo, Indonesia and Japan): the shape of the male aedeagi were identical, the chaetotaxy of the body and legs were the same and the shape of the peritremes and the spinning eupathidia were also identical.

Nucleotide variation

Complete sequences of ribosomal ITS2 were obtained for the seven samples of the *Tetranychus* complex collected from five different regions of the world. The ITS2 sequence of the *T. kanzawai* sample collected on tea in Japan is shown in fig. 1. The sequence has been deposited in the European Molecular Biology Laboratory (EMBL) database under the accession number X99876. No intra-strain polymorphism was detected in any of the samples examined. The length of ITS2 was 481 nucleotides. The ITS2 sequences displayed a high degree of similarity between mites collected from different geographic regions. The alignments of the seven sequences analysed displayed only three variable sites. These involved a transversion and a transition in positions 58 and 97 respectively of the sequence of the Australian strain and a transversion in position 21 of the sequence of the Indonesian strain. Given the similarity of the sequences analysed, the nucleotide alignments are obvious and are not shown here.

Crosses

All the crosses performed are presented in table 2. In conspecific crosses, egg hatchability was greater than 94% on average, and more than 73% of hatchlings that reached maturity were females. The survival rate in the juvenile stages was more than 96%. There was no reduction in the hatchability of eggs and in the survival rate of immatures for all heterospecific crosses compared with conspecific crosses. The sex ratio was slightly reduced in *T. hydrangeae* (Th) female and *T. kanzawai* (Tk) male crosses, but it was drastically reduced in *T. hydrangeae* female and *T. parakanzawai* (Tp) male crosses. In arrhenotokous species such as spider mites, unfertilized eggs develop into haploid males whereas diploid females develop from fertilized eggs. The reduction in sex ratio observed in the above experimental crosses suggests an increase in non-fertilized eggs. No female offspring appeared in the crosses between *T. parakanzawai* females and males of either *T. hydrangeae* or

Table 1. Collection sites of mites.

Localities	Plant	Supplier of collection	Collected
Sydney, Australia	*Hydrangea* sp.	M. Williams	1998
Brazzaville, Congo	*Manihot esculenta*	O. Bonato	March 1992
Indonesia	*Manihot esculenta*	P.A.V.D. Laan	November 1978
Tsukuba, Ibaraki, Japan	*Hydrangea macrophylla*	T. Gotoh	March 1993
Kanaya, Shizuoka, Japan	*Camellia sinensis*	M. Mochizuki	May 1993
Japan	*Pyrus communis*	E.E. Lindquist	October 1997
San Francisco, CA, USA	*Hydrangea* sp.	J. Gutierrez	September 1998

```
                                                                    50
GTTGAGATGTAAAATAATCAACAAAACACTTGCATACTACCATATATGCA
                         *
                                                                   100
TTGTTTTTAGAGGATTGTATATTTATATACATGAATCTTGATGTTTTATT
        *                                                     *
                                                                   150
CCTTTTCTTAATTGCAATTCGTTGCAATTTAGTAAGGAGAATCTCAAATC

                                                                   200
TACTTGTTTCACATGATAAATTTTGTGTACAATGCATATTTCATCTCTGC

                                                                   250
AAGCAGTATATATGAATAGATACTAGCATGAGATTCTAAGGTTAGTCGCC

                                                                   300
TATCTGACGACGCTAAAGTCGTATTGCAGATAACTATGGTGATCAACTAA

                                                                   350
CCTGTTAAATGATGAATCTTCTTGCACTTGTATAAATCATACAAATAGTA

                                                                   400
GCTATTTCATTCTGTTAAAGCAGACCTAAGAAGTAATGCAAAGGCAAAAT

                                                                   450
TTGTGTAAACGTTAAAGTAGATTTACGTTGCTTGCTTGCAAACAACACAA

                                                  481
ATATACATTAATCAACTTAATCAATATTTTT
```

Fig. 1. Sequences 5′ to 3′ of the full ITS2 ribosomal DNA of *Tetranychus kanzawai* collected on tea in Japan. Variable positions among six other origins analysed (see text) are shown by asterisks. EMBL database accession number X99876.

T. kanzawai. In the backcrosses between hybrid (*T. hydrangeae* × *T. kanzawai*) and (*T. kanzawai* × *T. hydrangeae*) females and males of either *T. hydrangeae* or *T. kanzawai*, the hatchability of eggs was reduced, especially in the cross between (*T. hydrangeae* × *T. kanzawai*) females and *T. kanzawai* males. A significant reduction in sex ratio was also observed. When (*T. hydrangeae* × *T. parakanzawai*) females were crossed with males of either *T. hydrangeae* or *T. parakanzawai*, female offspring was not produced. As reported by Gomi & Gotoh (1996), no females were produced when (*T. kanzawai* × *T. parakanzawai*) females were mated with *T. kanzawai* males, whereas when (*T. kanzawai* × *T. parakanzawai*) females were mated with *T. parakanzawai* males, female progenies were produced with a drastic reduction in egg hatchability (table 2).

Discussion

No reliable differences in morphological characters were detected between the samples of the *T. kanzawai–T. hydrangeae* complex examined in the present study. Comparison of the ribosomal ITS2 sequences obtained for the seven *Tetranychus* samples analysed and belonging to the *T. kanzawai–T. hydrangeae* complex revealed strong similarity since only one mutation was detected in each of the sequences for the individuals collected in Indonesia and two in those from Australia. This differentiation represents 0.4% of maximum nucleotidic divergence. As the error in *Taq* polymerase is of the same order (Cline *et al.*, 1996), complete similarity of all the seven sequences cannot be excluded. In any case, the nucleotidic divergence detected between morphologically close species, such as *T. pacificus* McGregor and *T. mcdanieli* McGregor, is 2.5% (Navajas *et al.*, 1997). The present study of the ITS2 sequence thus suggests conspecificity of the mites collected on *Hydrangea* spp. with those on other host plants.

An alternative way of addressing the question is to conduct breeding experiments to investigate reproductive compatibility between mites assumed to be *T. hydrangeae* and *T. kanzawai*. The present study shows that while crosses between *T. hydrangeae* and a closed related species, *T. parakanzawai*, were incompatible, there was potential for gene exchange between *T. hydrangeae* and *T. kanzawai*. Although weak reproductive incompatibility was observed between crosses of the latter species, crosses in both directions produced female offspring and compatibility was maintained in almost all cases in backcrosses (table 2). However, the fertility of the F1 males should be tested to fully resolve incompatibility patterns.

Partial reproductive incompatibility between conspecific populations in different localities or from different plants is a common phenomenon in spider mites (De Boer, 1985; Fry, 1989; Gotoh *et al.*, 1993, 1995; Gotoh & Tokioka, 1996; Gomi *et al.*, 1997). In the case of the green and red forms of *T. urticae* a drastic reduction was observed in the sex ratio, and 4–61% of F1 females did not oviposit when they were backcrossed with males from respective stock cultures

Table 2. Number of eggs laid during the first five days of oviposition, hatchability, survival rate of immatures and sex ratio of progeny from crosses among *Tetranychus hydrangeae* (Th), *T. kanzawai* (Tk) and *T. parakanzawai* (Tp).

Crossing female	×	male	N^1	No. of eggs per female	Hatchability (%)	Survival rate in immature stages (%)	% female offspring	Average no. of female offspring
Th		Th	19	41.3 ± 3.2^{abcde}	99.7 ± 0.2^{a}	96.1 ± 1.0	73.1 ± 1.8^{bcde}	29.4 ± 2.6^{ab}
Tk		Tk	23	41.0 ± 1.2^{abcde}	94.9 ± 1.3^{a}	96.1 ± 0.8	80.1 ± 1.1^{abc}	30.1 ± 1.0^{ab}
Tp		Tp	34	43.9 ± 1.2^{abcd}	99.2 ± 0.4^{a}	98.6 ± 1.1	82.8 ± 0.5^{ab}	35.5 ± 1.1^{a}
Th		Tk	30	33.4 ± 2.1^{cd}	97.0 ± 1.2^{a}	93.6 ± 1.9	61.3 ± 2.6^{e}	18.9 ± 1.8^{cd}
Tk		Th	22	38.5 ± 3.7^{bcde}	98.1 ± 0.7^{a}	94.7 ± 0.8	76.8 ± 1.8^{abcd}	27.5 ± 2.8^{abc}
Th		Tp	16	30.2 ± 3.0^{d}	98.9 ± 0.7^{a}	97.6 ± 0.8	2.0 ± 1.7^{f}	0.7 ± 0.6^{f}
Tp		Th	18	48.0 ± 2.2^{abc}	96.6 ± 1.0^{a}	95.9 ± 0.7	0^{f}	0^{f}
†Tk		Tp	21	37.3 ± 1.0^{bcde}	98.8 ± 0.5^{a}	99.1 ± 0.8	72.6 ± 1.3^{bcde}	26.5 ± 0.9^{abc}
†Tp		Tk	19	36.6 ± 0.8^{bcd}	99.1 ± 0.5^{a}	99.4 ± 0.4	0^{f}	0^{f}
(Th×Tk)		Th	22	49.5 ± 2.3^{ab}	72.5 ± 3.6^{b}	96.4 ± 0.9	88.6 ± 2.5^{a}	31.3 ± 2.5^{ab}
(Th×Tk)		Tk	23	38.7 ± 3.3^{bcde}	25.4 ± 3.0^{d}	95.6 ± 17	63.4 ± 5.7^{de}	6.7 ± 1.3^{ef}
(Tk×Th)		Tk	21	52.0 ± 1.7^{a}	64.4 ± 4.3^{b}	96.9 ± 1.6	73.5 ± 3.9^{bcde}	24.8 ± 2.7^{bc}
(Tk×Th)		Th	21	48.3 ± 2.5^{bcde}	75.4 ± 4.4^{b}	96.1 ± 2.0	79.3 ± 3.1^{abc}	28.6 ± 2.6^{ab}
(Th×Tp)		Th	10	43.5 ± 5.7^{abcd}	3.6 ± 1.7^{e}	95.0 ± 5.0	0^{f}	0^{f}
(Th×Tp)		Tp	5	44.0 ± 2.8^{abcd}	1.4 ± 0.6^{e}	100	0^{f}	0^{f}
†(Tk×Tp)		Tk	22	38.9 ± 0.8^{bcde}	35.7 ± 3.7^{cd}	99.0 ± 0.5	0^{f}	0^{f}
†(Tk×Tp)		TD	20	33.4 ± 1.0^{cd}	47.9 ± 3.2^{c}	98.1 ± 0.7	65.9 ± 2.2^{cde}	10.4 ± 0.9^{de}
F values[2]				7.067^{***}	149.753^{***}	1.827^{ns}	172.564^{***}	59.668^{***}

[1]Number of pairs examined.
[2]Means differ significantly at 0.1% (***) (multivariate ANOVA); ns not significant at 5%. Data are shown as mean ± S.E. Values in a column followed by different letters are significantly different ($P < 0.05$; Tukey–HSD test).
†After Gomi & Gotoh (1996).

(Gotoh & Tokioka, 1996; Gotoh *et al.* 1999a). Ovipositing F1 females produced fewer eggs than intra-form crosses, and the hatchability of B1 eggs showed a drastic decline. As a result, the average number of female offspring (B1) produced was less than two for each female (Gotoh *et al.* 1999a). Despite the reported reproductive incompatibility, the red and green forms of *T. urticae* are considered to be conspecific by many researchers (Dupont, 1979; De Boer, 1985; Gotoh *et al.*, 1993; Navajas, 1998), because fertile female offspring are produced bidirectionally in succeeding generations.

In our study some reduction in egg hatchability and an altered sex ratio were observed between *T. hydrangeae* and *T. kanzawai* crosses (table 2). Due to the large distance between the collection sites of these mites (Australia and Japan), the effect of geographical isolation cannot be excluded. Gotoh *et al.* (1999b), who studied *T. kanzawai* populations collected from a whole range of Japanese islands, showed that crosses between geographically distant populations resulted in a slight reduction in egg hatchability and sex ratio in offspring.

Both crossing experiments and DNA analysis results presented here are in agreement. On the one hand, *T. hydrangeae* and *T. kanzawai* mites are reproductively compatible and on the other, ribosomal ITS2 sequences revealed that samples from five different countries, including Japan, with a sample from tea, and a Californian sample from *H. macrophylla*, were practically identical. It can be concluded therefore that *T. hydrangeae* Pritchard & Baker is synonymous with *T. kanzawai* Kishida, as proposed by Wainstein in 1960.

The prevalence of the mite and the diversity of its host plants in numerous countries in South-east Asia lead to the conclusion that *T. kanzawai* originated in this region. In most of the other countries, such as the USA, Australia and South Africa, *T. kanzawai* is reported above all on *Hydrangea* spp., and it is probable that this tetranychid mite was introduced into these regions on these plants, that originated in China and are now widely distributed around the world as ornamentals. The hard winters in temperate countries may limit the mite to greenhouse crops and to its initial host plant. Thus it is likely that the species has disappeared or is only a temporary colonizer in France for example. It appears to have a serious economic impact in South-east Asia and many cases of resistance to several types of acaricide have been reported (reviewed in Goka, 1998). Its presence in a cassava plantation in Brazzaville, near to ornamental plants, and its absence in plantations remote from the capital of the Congo suggest that the mite was introduced there at a recent date, but may also represent a potential danger for tropical crops in general.

Many exotic pests now have a much greater opportunity of colonizing new areas as a result of the increasing movement of plants as part of the international horticultural trade. The unambiguous identification of any potential pest is a crucial step in quarantine procedures. DNA based methods that complement morphological studies, are a valuable tool for species diagnostics, especially if only few characters are available to distinguish between taxa as for many closely related species of spider mite (e.g. Gotoh *et al.*, 1998).

Acknowledgements

The authors thank H.R. Bolland (University of Amsterdam, The Netherlands), O. Bonato (Institut de Recherches pour le Développement, Montpellier, France) and E.E. Lindquist (Agriculture Canada, Ottawa, Canada) for kindly providing specimens for this study.

References

Baker, E.W. & Tuttle, D.M. (1994) *A guide to the spider mites (Tetranychidae) of the United States*. West Bloomfield, Michigan, Indira Publisher House.

Berlocher, S.H. (1999) Host race or species? Allozyme characterization of the 'flowering dogwood fly', a member of the *Rhagoletis pomonella* complex. *Heredity* **83**, 652–662.

Bolland, H.R., Gutierrez, J. & Flechtmann, C.H.W. (1998) *World catalogue of the spider mite family (Acari: Tetranychidae), with references to taxonomy, synonymy, host plants and distribution*. Leiden, Brill Academic Publishers.

Cline, J., Braman, J.C. & Hogrefe, H.H. (1996) PCR fidelity of pfu DNA polymerase and other thermostable DNA polymerases. *Nucleic Acids Research* **15**, 3546–3551.

De Boer, R. (1985) Reproductive barriers. *in* pp. 193–200 Helle W. & Sabelis M.W. (*Eds*) *World crop pests. Spider mites: their biology, natural enemies and control*, vol. IA. Amsterdam, Elsevier Science Publishers.

Dupont, L. (1979) On gene flow between *Tetranychus urticae* Koch, 1836 and *Tetranychus cinnabarinus* (Boisduval) Boudreaux, 1956 (Acari: Tetranychidae): synonomy between the two species. *Experimental and Applied Acarology* **25**, 297–303.

Ehara, S. (1956) Tetranychoid mites of mulberry in Japan. *Journal of the Faculty of Science, Hokkaido University (series VI) Zoology* **12**, 499–510.

Ehara, S. (1999) Revision of the spider mite family Tetranychidae of Japan (Acari, Prostigmata). *Species Diversity* **4**, 63–141.

Ehara, S. & Lee, L.H.Y. (1971) Mites associated with plants in Hong Kong. *Journal of the Faculty of Education, Tottori University, Natural Sciences* **22**, 61–78.

Ehara, S. & Masaki, M. (1989) Notes on two Japanese species of *Tetranychus* (Acarina: Tetranychidae). *Acta Arachnologica* **38**, 49–54.

Ehara, S. & Tho, Y.P. (1988) Spider mites of the Malay Peninsula (Acarina:Tetranychidae). *Journal of the Faculty of Education, Tottori University, Natural Sciences* **37**, 1–24.

Ehara, S. & Wongsiri, T. (1975) The spider mites of Thailand (Acarina: Tetranychidae). *Mushi* **48**, 149–185.

Fenton, B., Malloch, G., Jones, A.T., Amrine Jr, J.W., Gordon, S.C., A'Hara, S., McGavin, W.J. & Birch, A.N.E. (1995) Species identification of *Cecidophyopsis* mites (Acari: Eriophyidae) from different *Ribes* species and countries using molecular genetics. *Molecular Ecology* **4**, 383–387.

Fritz, G.N., Conn, J., Cockburn, A. & Seawright, J. (1994) Sequence analysis of the ribosomal DNA internal transcribed spacer 2 from a population of *Anopheles nuneztovari* (Diptera: Culicidae). *Molecular Biology and Evolution* **11**, 406–416.

Fry, J.D. (1989) Nuclear-nuclear and nuclear-cytoplasmic interactions contribute to the reproductive incompatibility between two strains of the twospotted spider mite. *Entomologia Experimentalis et Applicata* **50**, 97–100.

Goka, K. (1998) Mode of inheritance of resistance to three new acaricides in the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Experimental and Applied Acarology* **22**, 699–708.

Gomi, K. & Gotoh, T. (1996) Host plant preference and genetic compatibility of the Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Applied Entomology and Zoology* **31**, 417–425.

Gomi, K., Gotoh, T. & Noda, H. (1997) *Wolbachia* having no effect on reproductive incompatibility in *Tetrancyhus kanzawai* Kishida (Acari: Tetranychidae). *Applied Entomology and Zoology* **32**, 485–490.

Gotoh, T. & Tokioka, T. (1996) Genetic compatibility among diapausing red, non-diapausing red and diapausing green forms of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). *Japanese Journal of Entomology* **64**, 215–225.

Gotoh, T., Bruin, J., Sabelis, M.W. & 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. *Entomologia Experimentalis et Applicata* **68**, 171–178.

Gotoh, T., Oku, H., Moriya, K. & Odawara, M. (1995) Nucleus-cytoplasm interactions causing reproductive incompatibility between two populations of *Tetranychus quercivorus* Ehara & Gotoh (Acari: Tetranychidae). *Heredity* **74**, 405–414.

Gotoh, T., Gutierrez, J. & Navajas, M. (1998) Molecular comparison of the sibling species *Tetranychus pueraricola* Ehara & Gotoh and *T. urticae* Koch (Acari: Tetranychidae). *Entomological Science* **1**, 55–57.

Gotoh, T., Sugasawa, J. & Nagata, T. (1999a) Reproductive compatibility of the two-spotted spider mite (*Tetranychus urticae*) infected with *Wolbachia*. *Entomological Science* **2**, 289–295.

Gotoh, T., Gomi, K. & Nagata, T. (1999b) Incompatibility and host plant differences among populations of *Tetranychus kanzawai* Kishida (Acari: Tetranychidae). *Applied Entomology and Zoology* **34**, 551–561.

Gutierrez, J. & Schicha, E. (1983) The spider mite family Tetranychidae (Acari) in New South Wales. *International Journal of Acarology* **9**, 99–116.

Hillis, D.M. & Dixon, M.T. (1991) Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology* **66**, 411–429.

Kishida, K. (1927) Notes on *Tetranychus kanzawai* n. sp. a new tetranychid mite injurious to leaves of the mulberry tree in Japan. *Zoological Magazine* **39**, 105–107.

Marinucci, M., Romi, R., Mancini, P., Di Luca, M. & Severini, C. (1999) Phylogenetic relationships of seven palaearctic members of the *maculipennis* complex inferred from ITS2 sequence analysis. *Insect Molecular Biology* **8**, 469–480.

Meyer, K.K.P. (1974) A revision of the Tetranychidae of Africa (Acari) with a key to the genera of the world. *Department of Agricultural Technical Services, Republic of South Africa, Entomology Memoirs* **36**, 1–291.

Meyer, M.K.P.S. (1987) African Tetranychidae (Acari: Prostigmata) with reference to the world genera. *Department of Agriculture and Water Supply, Republic of South Africa, Entomology Memoirs* **69**, 1–175.

Navajas, M. (1998) Host plant associations in the spider mite *Tetranychus urticae* (Acari: Tetranychidae): insights from molecular phylogeography. *Experimental and Applied Acarology* **22**, 201–214.

Navajas, M., Gutierrez, J., Bonato, O., Bolland, H.R. & Mapangou-Divassa, S. (1994) Intraspecific diversity of the cassava green mite *Mononychellus progresivus* (Acari: Tetranychidae) using comparisons of mitochondrial and nuclear ribosomal DNA sequences and cross-breeding. *Experimental and Applied Acarology* **18**, 351–360.

Navajas, M., Gutierrez, J. & Gotoh, T. (1997) Convergence of molecular and morphological data reveals phylogenetic information in *Tetranychus* species and allows the restoration

of the genus *Amphitetranychus* (Acari: Tetranychidae). *Bulletin of Entomological Research* **87**, 283–288.

Navajas, M., Lagnel, J., Gutierrez, J. & Boursot, P. (1998) Species wide homogeneity of nuclear ribosomal ITS2 sequences in the spider mite *Tetranychus urticae* contrasts with extensive mitochondrial COI polymorphism. *Heredity* **80**, 742–752.

Navajas, M., Gutierrez, J., Lagnel, J., Fauvel, G. & Gotoh, T. (1999) DNA sequences and cross-breeding experiments in the hawthorn spider mite *Amphitetranychus viennensis* reveal high genetic differentiation between Japanese and French populations. *Entomologia Experimentalis et Applicata* **90**, 113–122.

Navajas, M., Tsagkarakou, A., Lagnel, J. & Perrot-Minnot, M.J. (2000) Genetic differentiation in *Tetranychus urticae* (Acari: Tetranychidae: polymorphism, host races or sibling species? *Experimental and Applied Acarology* in press.

Paskewitz, S.M., Wesson, D.M. & Collins, F.H. (1993) The internal transcribed spacers of ribosonal DNA in five members of the *Anopheles gambiae* species complex. *Insect Molecular Biology* **2**, 247–257.

Pritchard, A.E. & Baker, E.W. (1955) A revision of the spider mite family Tetranychidae. *Pacific Coast Entomological Society. Memoirs Series* **2**, 1–472.

Schicha, E. & Gutierrez, J. (1985) Phytoseiidae of Papua New Guinea, with three new species, and new records of Tetranychidae (Acari). *International Journal of Acarology* **11**, 173–181.

Shufran, K.A., Burd, J.D., Anstead, J.A. & Lushai, G. (2000) Mitochondrial DNA sequences divergence among greenbug (Homoptera: Aphididae) biotypes: evidence for host-adapted races. *Insect Molecular Biology* **9**, 179–184.

Wainstein, B.A. (1960) Tetranychoid mites of Kazakhstan (with revision of the family) (in Russian). *Trudy Nauchno-Isled., Instituta Zashchiti Rastenii, Kazakhstan Akademia Sel'sk Nauch.* **5**, 1–276.

(Accepted 25 October 2000)