Don Au

# Molecular Comparison of the Sibling Species *Tetranychus puerariicola* Ehara et Gotoh and *T. urticae* Koch (Acari: Tetranychidae)

Tetsuo GOTOH, Jean GUTIERREZ and Maria NAVAJAS



*Reprinted from*

Entomological Science, Vol. 1, No. 1, pp. 55–57. March 25, 1998

*The Entomological Society of Japan*

Entomological Science, 1998, 1(1): 55–57

# Molecular Comparison of the Sibling Species *Tetranychus puerariicola* Ehara et Gotoh and *T. urticae* Koch (Acari: Tetranychidae)

Tetsuo GOTOH[1], Jean GUTIERREZ[2] and Maria NAVAJAS[2]

[1]*Laboratory of Applied Entomology and Zoology, Faculty of Agriculture, Ibaraki University, Ami, Ibaraki, 300–0393 Japan*
[2]*Laboratoire de Zoologie, INRA-ENSAM-ORSTOM, Place P. Viala, 34060 Montpellier, France*

**Abstract.** DNA sequence data were used to examine relationships between the two sibling species *Tetranychus puerariicola* Ehara et Gotoh and *Tetranychus urticae* Koch. The second internal transcribed spacer (ITS2) of the ribosomal DNA was amplified by PCR and sequenced. The ITS2 sequences of these two species displayed a high degree of identity (98.3%) comparable to the nucleotide divergence observed in other pairs of closely related species. These data confirm the species status of *T. puerariicola* and are in agreement with previous results based on morphological analyses and crossing experiments. Furthermore, restriction-site analysis of the ITS2 region was performed in order to detect species-specific diagnostic restriction sites. Thus, the efficiency of molecular markers as a tool for determining the taxonomic status in spider mites was shown.

**Key words:** *Tetranychus puerariicola*, *Tetranychus urticae*, sibling species, ITS2, ribosomal DNA, PCR/RFLP.

## Introduction

*Tetranychus puerariicola* Ehara et Gotoh is considered to be a sibling species of *Tetranychus urticae* Koch (Ehara & Gotoh, 1996). Although one of the diagnostic characters, the shape of the aedeagal terminal knobs, is very similar in the two species, *T. puerariicola* may be distinguished from *T. urticae* by different morphological and biological characters such as the length of the terminal knob of the aedeagus, the shape of the lobes on the dorsal hysterosomal striae of females, and the appearance of feeding scars on host leaves. Crosses between *T. urticae* and *T. puerariicola* unidirectionally produce $F_1$ female adults which are either sterile or lay only non-viable eggs (Gotoh & Tokioka, 1996), so that the two species are incompatible with each other.

Such interspecific crosses have often been used to confirm the species status. Several authors (Newcomer, 1954; Helle & van de Bund, 1962; Smith, 1975; Jordaan, 1977; Hill & O'Donnell, 1991) attempted to cross 13 different *Tetranychus* species in different combinations. However, no hybridization occurred except in crosses between *Tetranychus hydrangeae* Pritchard et Baker and *T. urticae*, in which a few sterile females were produced (Helle & van de Bund, 1962).

*Tetranychus puerariicola* may be considered to be morphologically and genetically very close to *T. urticae* and so experience is required to separate these two species using morphological traits. Advances in molecular biology, in particular the comparison of nucleotide sequences of specific DNA regions amplified by the polymerase chain reaction (PCR) followed by either restriction fragment length polymorphism (RFLP) analysis or direct sequencing of PCR products, may help to distinguish between closely related species. The ribosomal DNA (rDNA) genes have been recognized to be useful for assessing the relationships between species: rDNA is highly repetitive and contains variable regions flanked by more conserved regions (Hillis & Dixon, 1991). The first property enhances PCR amplification because many templates are available for initial priming, whereas the second allows primers to be designed for conserved regions, which are the coding regions, and to amplify the variable regions, i.e., the two internal transcribed spacers (ITS1 and ITS2). Ribosomal DNA sequences are thus becoming a general tool for both taxonomic and phylogenetic studies. In addition, ITS sequence data have been used for distinguishing species of arthropods, mainly in insects (Wesson *et al.*, 1992; Campbell *et al.*, 1993; Fritz *et al.*, 1994), and they have also proved to be useful in solving spider mite affiliations (Navajas *et al.*, 1992, 1994, 1997) and for research on eriophyid mites (Fenton *et al.*, 1995) and ixodid ticks (Wesson *et al.*, 1993; McLain *et al.*, 1995).

In the present study, we analyzed variations in nucleotide sequences in the second internal transcribed spacer, ITS2, of the ribosomal DNA in *T. puerariicola* and *T. urticae* in order to better understand the relationship between these sibling species.

## Materials and Methods

Specimens of *T. puerariicola* were collected at Hitachi-Ohta, Ibaraki, on kudzu vine, *Pueraria lobata* (Willd.). DNA was isolated from single individuals and an aliquot of the extracted DNA was used as a template for PCR amplification of the ribosomal ITS2. The primers used were defined in the flanking 5.8S and 28S regions. Extraction and PCR methods, primers and subsequent direct sequencing procedures were performed as described in detail by Navajas *et al.* (1997). Four individuals were treated separately for assessment of intrastrain variation.

The ITS2 sequence obtained for *T. puerariicola* was compared

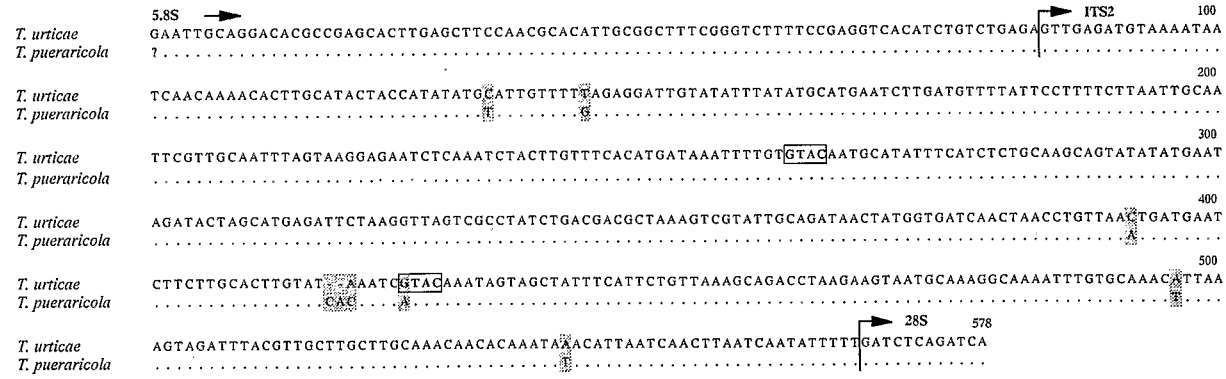


Fig. 1. ITS2 sequences 5' to 3' of *Tetranychus urticae* and *Tetranychus puerariculo*. Flanking sequences corresponding to 5.8S and 28S genes are also included. Dots signify that the sequence matches whereas dashes represent insertion/deletions. Differences between both sequences are shaded and the recognition sites for the restriction enzyme *Rsa* I are boxed.

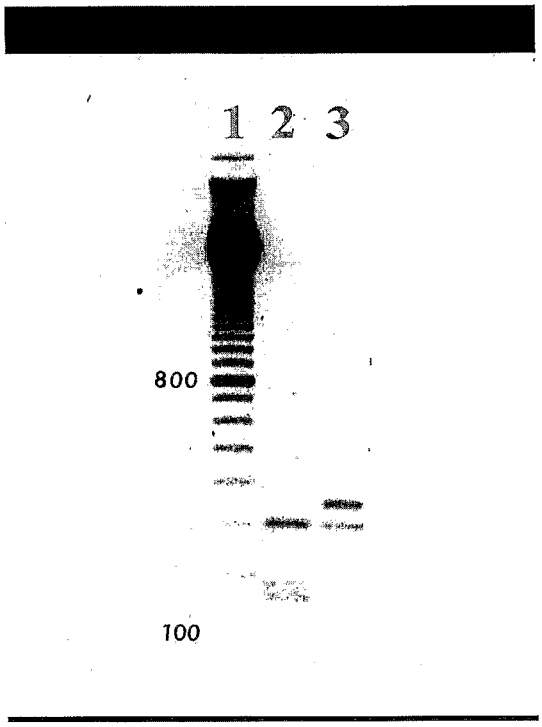


Fig. 2. Restriction fragment length differences of ITS2 sequences of two tetranychid species. PCR-amplified products derived from a single mite DNA were digested with the restriction enzyme *Rsa* I. Lane 1: size marker, 100 bp ladder; lane 2: *Tetranychus urticae*; lane 3: *T. puerariculo.*

to that previously obtained for *T. urticae* based on 18 samples collected worldwide on a variety of host plants (Navajas *et al.*, unpublished data).

For RFLP analysis, the PCR products obtained for both species were digested by the enzyme *Rsa*I and the resulting fragments were separated by electrophoresis on a 1.2% agarose gel and stained with ethidium bromide.

## Results and Discussion

The alignment of the nucleotide sequences of the rDNA ITS2 and flanking 5.8S and 28S regions of *T. puerariculo* and *T. urticae* is shown in Fig. 1. The sequences have been deposited in the EMBL database under the accession number X99881 and Y13609 for *T. urticae* and *T. puerariculo*, respectively. The boundaries of the ITS2 were determined by homology stretches in the two flanking regions as classically displayed by those sequences. No intrastrain polymorphism was detected in the ITS2 sequences of the samples. The length of the ITS2 sequences of *T. urticae* and *T. puerariculo* was 481 and 483 nucleotides, respectively. The two ITS2 sequences displayed a high degree of identity (98.3%) and the differences observed involved 7 point mutations (2 transitions and 5 transversions) and an insertion/deletion.

Comparison with data obtained for other closely related tetranychid species is needed for a full explanation of the sequence divergence between *T. urticae* and *T. puerariculo.* Similar nucleotide divergences have been observed in other comparisons between morphologically similar species such as *T. urticae* and *T. kanzawai* Kishida or *T. mcdanieli* McGregor and *T. pacificus* McGregor. The ITS2 sequences were 98.8% homologous in the former pair and 97.5% homologous in the latter pair (Navajas *et al.*, 1997). These data support the species status of *T. puerariculo* and are consistent with the previous results of morphological studies and cross-breeding tests.

However, nucleotide sequencing is expensive and laborious, and thus a quick species-diagnostic test based on RFLP techniques would be of great value. It is possible to find differences between species in the ITS2 sequences as presented here. The recognition site for *Rsa* I (GTAC) was observed at positions 177–180 and 341–344 in the *T. urticae* ITS2 sequence (Fig. 1), while it was present only at the former position in the *T. puerariculo* sequence. In addition, a mutation at position 413 in the *T. urticae* sequence disrupts a recognition site of the enzyme *Dra* I (TTTAAA) that is present in *T. puerariculo.* Both sites can therefore be used to discriminate between the two mite species. The potential of the restriction fragment length differences in the ITS2 for distinguishing the two species is illustrated in Fig. 2. In this figure, *Rsa* I digestion of the PCR-amplified ITS2 derived from a single mite DNA clearly shows that the *T. urticae* sequence produces 3 fragments (implying 2 recog nition sites), while the *Rsa* I restriction profile of the ITS2 sequence of *T. puerariculo* yields only 2 fragments (implying a single recognition site).

Our present approach is based on the fact that the sequences obtained are homogeneous for the species. Intraspecific variation in *T. urticae* has been extensively investigated in a worldwide survey including 18 samples from four continents, and the results indicate homogeneity of the ITS2 sequence in these species (Navajas *et al.*, unpublished data). Polymorphism of the ribosomal spacer sequences has nevertheless been detected in some arthropods (Vogler & De Salle, 1994) including spider mites (Navajas *et al.*, 1994). A test relying on restriction site differences between the two species postulates that the ITS2 sequence in *T. puerariсola* is not polymorphic, at least in the *Rsa* I and *Dra* I target positions. This point requires further confirmation.

The results presented here confirm the species status of *T. puerariсola* by discriminating it from *T. urticae*. We have shown that the molecular approach is useful for solving taxonomic problems when diagnostic morphological traits are scarce. Although species may be usually identified by morphological characteristics, it is important to confirm by molecular means if slight morphological differences are reliable for distinguishing species.

## References


Campbell, B. C., Steffen-Campbell, J. D. & Werren, J. W. 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect Molecular Biology,* **2**: 225–237.

Ehara, S. & Gotoh, T. 1996. Two new species of spider mites occurring in Japan (Acari, Tetranychidae). *Journal of the Acarological Society of Japan,* **5**: 17–25.

Fenton, B., Malloch, G., Jones, A. T., Amrine Jr., J. W., Gordon, S. C., A'Hara, S., McGavin, W. J. & Birch, 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 transcribe spacer 2 from population of *Anopheles nuneztovari* (Diptera: Culicidae). *Molecular Biology and Evolution,* **11**: 406–416.

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.

Helle, W. & van de Bund, C. F. 1962. Crossbreeding experiments with some species of the *Tetranychus urticae* group. *Entomologia Experimentalis et Applicata,* **5**: 159–169.

Hill, R. L. & O'Donnell, D. J. 1991. Reproductive isolation between *Tetranychus lintearius* and two related mites, *T. urticae* and *T. turkestani* (Acarina: Tetranychidae). *Experimental and Applied Acarology,* **11**: 241–251.

Hillis, D. M. & Dixon, M. T. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *The Quarterly Review of Biology,* **66**: 411–453.

Jordaan, L. C. 1977. Hybridization studies on the *Tetranychus cinnabarinus* complex in South Africa (Acari: Tetranychidae). *Journal of the Entomological Society of Southern Africa,* **40**: 147–156.

McLain, D. K., Wesson, D. M., Collins, F. H. & Oliver, J. H. 1995. Evolution of the rDNA spacer, ITS2, in the ticks *Ixodes scapularis* and *I. pacificus* (Acari: Ixodidae). *Heredity,* **75**: 303–319.

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

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.

Newcomer, E. J. 1954. Identity of *Tetranychus pacificus* and *mcdanieli. Journal of Economic Entomology,* **47**: 460–462.

Smith, J. W. 1975. Spider mites: population suppression by interspecific hybridization. *Environmental Entomology,* **4**: 588–590.

Vogler, A. P. & De Salle, R. 1994. Evolution and phylogenetic information content of the ITS-1 region in the tiger beetle *Cicindela dorsalis. Molecular Biology and Evolution,* **11**: 393–405.

Wesson, D. M., Porter, C. H. & Collins, F. H. 1992. Sequence and secondary structure comparisons of ITS rDNA in mosquitoes (Diptera: Culicidae). *Molecular Phylogenetics and Evolution,* **1**: 253–269.

Wesson, D. M., McLain, D. K., Oliver, J. H., Piesman, J. & Collins, F. H. 1993. Investigation of the validity of species status of *Ixodes dammnini* (Acari: Ixodidae) using rDNA. *Proceedings of the National Academy of Sciences of the United States of America,* **90**: 10221–10225.


(Received March 19, 1997; Accepted August 19, 1997)

**FICHE DESCRIPTIVE**

A joindre à tout envoi de document

à : Cellule de Collecte FDO
ORSTOM
70-74, route d'Aulnay
93143 BONDY CEDEX

Auteur(s) T. Gotoh ; J. Gutierrez ; M. Navajas 1998

Titre original : Molecular Comparison of the Sibling Species *Tetranychus puerariicola* Ehara et Gotoh and *T. urticae* Koch (Acari : Tetranychidae)

Titre en Français : (si le document est en langue étrangère) Comparaison par la biologie moléculaire des espèces soeurs *Tetranychus puerariicola* Ehara et Gotoh et *T. urticae* Koch (Acari : Tetranychidae)

ENTOMOL. SCI., Vol 1 (1) : 55-57.

Mots-clés : *Tetranychus puerariicola, Tetranychus urticae*, espèces soeurs, ITS2 , ADN ribosomal, PCR/RFLP

Key words : *Tetranychus puerariicola, Tetranychus urticae*, sibling species, ITS2, ribosomal DNA, PCR/RFLP

Résumé en français : Les résultats d'analyses de séquences d'ADN ont été utilisées pour examiner les relations entre les deux espèces soeurs *Tetranychus puerariicola* Ehara et Gotoh et *Tetranychus urticae* Koch. L'ITS2 de l'ADN ribosomal a été amplifié par PCR et séquencé. Les séquences de l'ITS2 de ces deux espèces présentent un haut degré d'identité (98, 3 %), comparable à la divergence nucléotidique notée dans les autres couples d'espèces très voisines. Ceci confirme le statut de *T. puerariicola* et est en accord avec les résultats précédement obtenus par les analyses morphologiques et les croisements. De plus une analyse des sites de restriction de l'ITS2 a été réalisée pour détecter leur spécificité. L'éfficacité des marqueurs moléculaires comme outil de détermination du statut spécifique des tétranyques est une fois de plus démontré.

Résumé en anglais : DNA sequence data were used to examine relationships between the two sibling species *Tetranychus puerariicola* Ehara et Gotoh and *Tetranychus urticae* Koch. The second internal transcribed spacer (ITS2) of the ribosomal DNA was amplified by PCR and sequenced. The ITS2 sequences of these two species displayed a high degree of identity (98.3 %) comparable to the nucleotide divergence observed in other pairs of closely related species. These data confirm the species status of *T. puerariicola* and are in agreement with previous results based on morphological analyses and crosssing experiments. Furthermore, restriction-site anlysis of the ITS2 region was performed in order to detect species-specific diagnostic restriction sites. Thus the efficiency of molecular markers as a tool for dtermining the taxonomic status in spider mites was shown.

Les titres, mots-clés matières et résumés en Anglais sont indispensables pour les documents destinés à entrer dans les Bases AGRIS et ASFA (Aquatic Sciences and Fisheries Abstracts)