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Title: Isolation and characterization of polymorphic microsatellite markers in *Tetranychus urticae* and cross amplification in other Tetranychidae and Phytoseiidae species of economical importance.

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Abstract

Tetranychus urticae Koch is a cosmopolitan phytophagous mite considered as the most polyphagous species among spider mites. This mite constitutes one of the key pests of clementine mandarins in the region of La Plana, where Spanish clementine production concentrates. Population genetic studies using molecular markers such as microsatellites have been proved to be extremely informative to address questions about population structure, phylogeography and host preferences. The aim of this study was to develop new microsatellite markers to differentiate *T. urticae* populations occurring in citrus orchards, in both the trees and weeds. Five different microsatellite DNA libraries were developed using probes with the motives CT, CTT, GT and CAC following the FIASCO protocol. Positive clones, those that included the insert with the microsatellite, were detected using the PIMA-PCR technique. On 22 of 32 new microsatellites loci combinations of primers were designed and their polymorphism was tested in four populations sampled along the eastern coast of Spain, obtaining 11 successful amplification. Cross amplification was tested in *T. turkestani, T. evansi, T. okinawanus, Panonychus citri, Eutetranychus orientalis, E. banksi*, *Oligonychus perseae* and *Aphlonobia histricina*, species belonging to the same Tetranychidae family, and in *Typhlodromus phialatus, Neoseiulus californicus, N. barkeri, Euseius stipulatus, Phytoseiulus persimilis, Amblyseius swirskii, A. cucumeris* and *A. andersoni* belonging to the Phytoseiidae family, obtaining 8 successful cross amplifications.

The final goal of our research was to increase the available molecular tools to gain insight into the genetic structure of *T. urticae* populations of citrus orchards, which might help in its management.

Keywords: *Citrus clementina*, microsatellites, SSRs, IPM

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is an important pest of citrus in Spain (Aucejo-Romero et. al. 2004; Ansaloni et al. 2007; Aguilar-Fenollosa et al. 2011) as well as in some citrus growing areas, especially on mandarins under Mediterranean climate (Bodenheimer, 1951; Talhouk, 1975; Swirski, 1977; McMurtry, 1985; Vacante, 1986; Hmimina et al. 1995; Souliotis et al. 1997). This mite constitutes the key pest of Clementine mandarins, *Citrus clementina* Hort. ex Tan., in the region of La Plana, the area around the city of Castelló de la Plana (39º 59'N; 00º 02'W), where Spanish Clementine production concentrates (around 1.5 10^6 tons; 60 10^3 ha). Mite infestations in Clementine varieties result in chlorotic spots on leaves, but more importantly, in fruit scarring, which decreases its commercial value. Our main goal is to study the genetic variation of this important pest in terms of host specialization, adaptation to different production systems (organic vs IPM), and phylogenetic relationships. The study of the genetic structure of populations of *T. urticae* in citrus groves appears as a powerful approach to estimate gene flow among mites infesting different plants in the agroecosystem. Different molecular techniques, such as microsatellite markers, isolated in *T. urticae* and other related mite species (Navajas et al. 1998a, 2000; Nishimura et al. 2003, Uesugi et al. 2007, Abercrombie et al. 2009 and Hinomoto et al. 2010), and the sequence of mitochondrial DNA gene coding for cytochrome oxidase I (COI) have already been used in tetranychid mites to study both inter- and intraspecific variation among populations (Navajas, 1998; Navajas et al. 1998b, 1999, 2000; Navajas and Fenton 2000; Hinomoto and Takafuji 2001; Tixier et al. 2002a,b; Bailly et al. 2004; Xie et al. 2006; Ben-David et al. 2007; Carbonelle et al. 2007; Uesugi et al. 2009a,b; Li et al. 2009). Microsatellite markers have become one of the most popular genetic markers and they have been chosen in ecological studies because of their high polymorphism.

The enormous adaptability of *T. urticae* to different host plants (Gould, 1979; Fry, 1989, 1992; Agrawal, 2000), and the fact that in the citrus agrosystem, this mite can be found feeding on many plant species (Aucejo et al. 2003; Aguilar-Fenollosa et al. 2011), have stirred our interest in determining the existence of host races of this species. Using the microsatellites developed by Navajas et al. (2002) in *T. urticae* populations of citrus orchards from Eastern Spain, we have found a low level of polymorphism in these populations. Only phylogeographic differences have been established in our populations (Hurtado et al. 2008a), with two *T. urticae* metapopulations: inland and coastal

populations. The massive use of acaricides in commercial citrus orchards may explain these results, which would result in genetic bottlenecks in founder populations and reduced genetic variability in the populations. The development of new microsatellites for *T. urticae* will increase the number of tools for genetic differentiation of mite populations, which may help in refining the management of this pest species. Moreover, these new microsatellite loci could prove useful in other economically important mite species like Phytoseiidae and other Tetranychidae. Bailly et al. (2004) used the *T. urticae* microsatellites developed by Navajas et al. (2002) in a closely related species, *Tetranychus turkestani*, and found geographical differences in this case, as well. Li at al. (2009) studied the genetic differences between *T. urticae* and *T. cinnabarinus* using the microsatellites developed by Navajas et al. (2002) and Uesugi and Osakabe (2007), having no amplification with the last ones loci, despite that in a more recent work the species status of *T. cinnabarinus* has been revised and assigned to the *T. urticae* species (de Mendoça et al. 2011). The genetic study of mite species can also address questions about mite genetic adaptation, which can be useful to understand the relationships between the mites present in a specific agroecosystem in order to develop suitable Biological Control strategies.

In summary, our goal is to develop microsatellite markers for mites using *T. urticae* as model species.

Materials and Methods

Unless otherwise indicated, all molecular techniques and solutions were performed as described by Sambrook et al. (1989).

Biological material

Tetranychus urticae Koch were collected from a laboratory colony maintained at the Entomology unit of IVIA (Valencia, Spain) which originated from the stock colony of Universitat Jaume I (UJI). This colony was started in 2001 from field collected material in the Castelló area.

Other mites (table 1) were field collected at different locations, individualized in eppendorf tubes and stored at -20ºC till DNA extraction.

DNA extraction

Tetranychus urticae total DNA was isolated following 'Salting out' protocol (Sunnucks and Hales, 1996) from a pool of 100 individuals (both sexes) for the generation of enriched libraries.

For testing the markers in all the species individualized extractions were performed following the same protocol.

Enriched library

T. urticae total DNA was enriched for microsatellite motifs CT, CTT, GT and CAC following the FIASCO protocol (Zane et al. 2002) using *Mse*I and *Alu*I as restriction enzymes for the genome fractionation. Enriched DNA fractions were cloned into pGEM-T easy (Promega Biotech Ibérica SL., Madrid, Spain) and transformed into DH5α electrocompetent *E. coli* cells (Invitrogen S.A., Barcelona, Spain) to obtain the libraries.

Library screening

Each library was plated on selective LB agar plates, white colonies were transferred to v-well plates containing 150 μl of liquid TB-glycerol medium with 50μg/ml ampiciline. Cultures were set at 37ºC, overnight without agitation. Cultured plates were stored at -80ºC till used. Plates were subjected to colony PCR (Sabater-Muñoz et al. 2006) and PIMA-PCR technique (Lunt et al. 1999) to select clones with microsatellite motif (Figure 1). Positive clones, those that have included the insert with the microsatellite, were reamplified with M13 universal primers, purified with Sephadex G-50 superfine (GE-Amersham Healthcare, Chalfont St. Giles, UK) and verified by gel electrophoresis on a 2% agarose gel (Pronadisa, Sumilab S.L., Madrid, España). Purified PCR products were directly sequenced at Servicio Central de Soporte a la Investigación Experimental (SCSIE) at Universitat de València using Bigdye® v3.1 chemistry (Applied Biosystems, Foster City, CA, USA) with primers T7 and SP6 in 1/16 of the recommended reaction volume.

Microsatellite markers design

Electropherograms were checked and assembled into consensus sequences by using Staden package software (Staden et al. 2003) for each clone. Each consensus sequence was compared to GenBank by blast using blastN as implemented in NCBI web page to compare with other mite sequences. The sequences reported in this work have been deposited into GenBank at NCBI (National Center for Biotechnology Information) under accession numbers GU339354 to GU339386.

After these comparisons, primers were designed in conserved regions of 21 microsatellite loci using Oligo software version v4.0 (Rychlik, 1991), amplification conditions were set up for each marker. Final successful conditions were 1x Taq pol buffer (Roche, Applied Science, Mannheim Germany), dNTPs, 2mM MgCl2, 0.2 µM primers, 1 u. Taq pol (Roche, Applied Science, Mannheim Germany) and 1µl of DNA template. Microsatellite makers were tested with ten individuals of the following populations: (1) the original *T. urticae* colony (2) Llíria (inland population; 39º38'N 0º36'W), (3) Callosa d'En Sarrià (coastal population; 38º39'05''N, 0º07'22''W), (4) Vinaròs (coastal population; 40º28'N 0º29'E) and (5) Inca (Mallorca Island; 39º43'N 2º54'E), as well as with 10 individuals from each of the species listed in Table 1. Thermal profile was: a first denaturation at 94ºC for 2 min, 40 cycles at 94ºc for 15 sec, 55-50ºC for 15 sec , and 72ºC for 15 sec, followed by a last cycle at 72ºC for 1 min, and a hold step at 4ºC. Amplification was performed in a PTC-200 thermal cycler (MJ Research, Bio-Rad Inc., Hercules, CA, USA). Several other manufactures (PCR reagents and thermocyclers) have been also tested positively.

Amplification was verified either by acrylamide gel electrophoresis with silver staining or, when forward primer was labelled with FAM-6 analyzed in an ABI/PE 3130 GeneAnalyzer (Applied Biosystems). Genotyping was performed using Peak Scanner v1.0 (Applied Biosystems 2006).

Results

Microsatellite markers design

The FIASCO enrichment performed resulted in a total of 1,786 independent clones isolated from the six cDNA libraries, from which 407 clones were PIMA-PCR selected and single-pass sequenced. After sequence confirmation of microsatellite presence, bidirectional sequencing was performed for 34 clones. Only 2 out of the 34 selected clones by PIMA methodology did not contain a microsatellite sequence (but contained several bi or tri-repeats of the motifs which could not be set as a real microsatellite), which means that this methodology is a 95% successful in microsatellite detection. Microsatellite distribution in libraries was 40 % and 34 % from CAC and CTT libraries, respectively. The remaining 26% corresponded to CT and GT libraries.

Blast comparisons showed that none of the clones obtained were already present in the data base. Only one clone produced a significant blast similarity with a mite sequence,

corresponding to a small microsatellite found in the 18S rDNA gene from *Hypochthonius rufulus* Koch, 1835 (Acari: Oribatida). From the remaining 32 clones, 17 contained a perfect microsatellite motif and 15 contained imperfect motifs. Microsatellite loci names, motif and designed primers are listed in table 2.

Amplification mismatches and other amplification patterns.

Three loci yielded amplicons of larger than the expected. These amplicons were directly sequenced with the corresponding primers. The sequence comparison revealed that the amplicon corresponded to different microsatellite loci. These new sequences have been deposited in GeneBank under code numbers XX and XX.

The forward primer designed for locus m14E02 was also found in other sequences of the loci described in this work, specifically in locus m11C09. Both loci were discarded as amplification pattern was of multiple polymorphic bands, a pattern described in this work as AFLP-like. Other loci with the same AFLP-like amplification pattern are listed in table 4.

Polymorphism detection on microsatellite markers.

The number of alleles for each microsatellite loci tested varied from 1 to 6, and are listed in Tables 2 and 3 for Tetranychidae and Phytoseiidae species, respectively.

Interspecific use of microsatellite markers

Designed markers were tested with the species listed in Table 1 for cross-amplification. Amplification conditions were the same as for the marker development, and no effort was undertaken to optimize amplification for unsuccessful or AFLP-like cross-amplification. Tables 2 and 3 summarize this cross reactivity, including size and allele number of each marker per species. In summary, 8 loci were successfully cross-amplified in other Tetranychidae species, and 7 in Phytoseiidae (Table 5).

Discussion

11 new microsatellite loci for the characterization of two spotted spider mite populations have been developed. These new loci have been sorted out from a library enrichment which resulted in a 95% successful microsatellite identification, an extremely higher percentage than that obtained in other works (Navajas et al. 1998, 2002; Nishimura et al. 2003; Uesugi and Osakabe 2007). Our results are in agreement with another work in which CT and GT motifs were less represented (Navajas et al. 1998). Simultaneously with our work, 16 new microsatellite loci for the same species

have been developed based on the CT and GT motifs (Uesugi and Osakabe 2007). In other closely related species, *T. kanzawai,* the motives CT and GT were also the targets for microsatellite characterization (Nishimura et al. 2003).

We have obtained a similar rate of loci discarding as other authors (Navajas et al. 2002, Nishimura et al. 2003 and Uesugi et al. 2007). We were able to design 22 combinations of primers from the 32 microsatellite clones obtained. However, after amplification only 11 microsatellite markers were selected. Li et al. (2009) used in their studies the microsatellites developed by Uesugi and Osakabe (2007) and got no amplification. Nevertheless, we have tested some of them (TuCA12, TuCA25, TuCA72, TuCA83, TuCA96, TuCT04, TuCT17, TuCT18, TuCT26, TuCT73 and TkMS015) in our populations (table 1) and obtained successful amplifications with at least two different alleles each. We have also included a population sampled in Florida (USA), which showed no amplification with our loci but which presented successful amplification in 9 of the 11 Japanese loci tested (Nishimura et al. 2003, Uesugi and Osakabe 2007) (data not shown). These results address questions about the difficulty in transferring the *T. urticae* microsatellite markers between continents. Despite that Japan and Spain belong to the Palaearctic Region, whereas USA belongs to the Nearctic Region, the results obtained with these primers show a close relationship between Japanese and USA *T. urticae* populations than between those in the same biogeographical region. This question appeared also when ITS (Hurtado et al. 2008) and COI gene (Navajas et al. 1998, Hinomoto et al. 2001) were analyzed and it deserves further research.

Microsatellite markers in *T. urticae* are hard to obtain. In the present study microsatellites were not only isolated, but also characterized and tested in populations of *T. urticae* originating from different areas of the Mediterranean Western coast, as well as in other mite species of economical importance, such as predatory Phytoseiidae mites. The transfer of markers to other species is a key point, since they can be useful for population studies, including detection of the origin of invasive species such as *Eutetranychus orientalis, E. banksi,* or *Olygonychus perseae,* which have been recently introduced in Spain.

The transference of microsatellites from the source species to closely related species is a subject of interest due to the difficulties and economical investment needed irrespective of the taxons studied (Bech et al. 2010, Canales-Aguirre et al. 2010, Olivatti et al. 2011 or Telles et al. 2011). In Tephritid flies, a species group of economical interest in agriculture as the mites used in this work, microsatellites are transferable between

species with variable degree, ranging from 49% to as less as 24% when species belong to different genera from the one of the source species (Augustinos et al. 2008). In this work, the authors reported a locus size mean difference of less than 50 bp, estimated by agarose gel electrophoresis, between species when considering the same locus. This degree of PCR product size conservation was variable and also linked to the relatedness of species. Although we obtained similar results of cross-species transferability, our results taking into account percentage of functional primers and expected size, are not indicative of the phylogenetic history of the mite species, as proposed with Tephritidae. We obtained a similar percentage of cross amplification in phytophagous and predatory mites, which keep far phylogenetic relationships (Navajas et al. 1998b, 1999). This close "clustering" of cross amplification may be related to a parallel evolution or co-evolution of phytophagous (prey) and entomophagous (predator) mites. In relation to this issue (predator-prey relationship) we made sure that the cross-amplification was true and not an artefact due to the presence of phytophagous DNA (prey) in the phytoseid gut (predator) by PCR based methods developed by our group (same authors, not yet published results).

The usefulness of the markers presented in this study is obvious both from a basic (e.g. population studies, assessment of predation, etc.) and an applied (e.g. quality control in commercial insectaries) points of view. As stated before, due to the difficulties and the high economical investment needed to develop species specific microsatellite markers, the transference of microsatellite markers among species (heterologous amplification) is a very interesting alternative.

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FIGURES and Tables LEGENDS

Fig. 1 Amplification pattern of clones containing microsatellites by using PIMA. First and last lanes correspond to molecular weight marker (100bp ladder, Invitrogen). Some lanes marked with a white arrow corresponded to clones containing microsatellites, as the two PCR products are formed by combination of universal M13 primers and microsatellite motif PIMA primer.

Table 1. Mites species used in this work

Table 2. Characteristics of loci microsatellite developed in *Tetranychus urticae* and cross amplification in other Tetranychidae species. Primer sequences for each locus are indicated for forward (for) and reverse (rev), with indication of the annealing temperature for each locus. For cross amplification, allele size range (in base pairs) and number of alleles (within brackets) are indicated.

Table 3. Characteristics of new *Tetranychus urticae* microsatellite loci in cross amplification in Phytoseiidae species. Allele size range (in base pairs) and number of alleles (within brackets) or amplification pattern are indicated.

Table 4. Loci discarded by amplification failure or multiple banding results (AFLP-like loci) in *Tetranychus urticae* samples

Table 5. Summary of cross-species amplification of *T. urticae* microsatellite markers in 17 mite species.

Fig. 1. Amplification pattern of clones containing microsatellites by using PIMA. First and last lanes correspond to molecular weight marker (100bp ladder, Invitrogen). Some lanes marked with a white arrow corresponded to clones containing microsatellites, as the two PCR products are formed by combination of universal M13 primers and microsatellite motif PIMA primer.

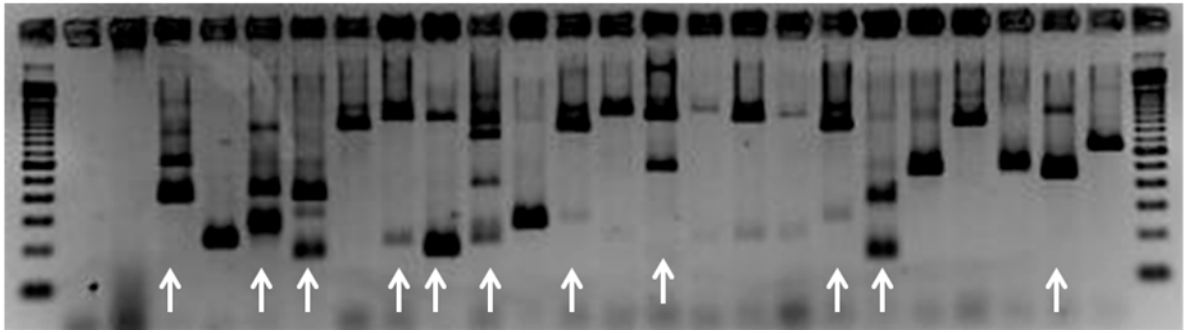

Table 1. Mites species used in this work

Family	Species	Code	Origin
Tetranychidae	*Tetranychus urticae*	TuCs	Castelló
	T. urticae	TuLi	Llíria
	T. urticae	TuMall	Inca
	T. urticae	TuVi	Vinaròs
	T. urticae	TuCa	Callosa d'En Sarrià
	T. turkestani	Tt	Almenara
	T. evansi	Te	Murcia
	T. okinawanus	To	Florida
	Panonychus citri	Pc	Moncada
	Eotetranychus orientalis	Eo	Málaga
	E. banksi	Eb	Huelva
	Oligonychus perseae	Op	Málaga
	Aplonobia histricina	Ah	Moncada
Phytoseidae	*Typhlodromus phialatus*	Tp	Moncada
	Neoseiulus californicus	Nc	Koppert Biological Systems
	N. barkeri	Nb	Castelló
	Euseius stipulatus	Es	Moncada
	Phytoseiulus persimilis	Pp	Koppert Biological Systems
	Amblyseius swirskii	As	Koppert Biological Systems
	A. andersoni	Aa	Biobest Biological Systems
	A. cucumeris	Ac	Koppert Biological Systems

Table 2. Characteristics of loci microsatellite developed in *Tetranychus urticae* and cross amplification in other Tetranychidae species. Primer sequences for each locus are indicated for forward (for) and reverse (rev), with indication of the annealing temperature for each locus. For cross amplification, allele size range (in base pairs) and number of alleles (within brackets) are indicated.

Locus	Motif	Primer sequences (5'→3')	Ann. T (ºC)	TuCS	TuLi	TuVi	TuCa	TuMall	Tt	Te	Pc	Eo	Eb	Op	Ah	Tok
M11b04	CAC	GAG GTT GTC AGT CAT CGT TTC (for) CGA TGA GTC CTG AGT AAT GAT (rev)	55	70-79 (4)	70-79 (4)	54-66 (5)	52-64 (5)	54 (1)	70-82 (5)	70-85 (5)	67-85 (6)	76-79 (2)	76 (1)	76-82 (2)	70-85 (5)	70-79 (4)
M11g09	CTT	ACC TAA AGA AGA CGA GCA AGA (for) AAA GCA GCA GAC ACA ACA AAT (rev)	55	122 (1)	122 (1)	122-146 (2)	122-146 (2)	122 (1)	122 (1)	156 (1)	90-99 (2)	-	122-146 (2)	110- 185 (4)	116-122 (2)	122 (1)
M11e03	CAC	ATT TTC CAC TGG ATG ACC TGG (for) CCT CTT CCT CCT CAT CAT CAC (rev)	55	182-203 (2)	182-203 (3)	203-215 (2)	190-203 (2)	203 (1)	203(1)	182 (1)	182-203 (2)	-	191-203 (2)	203 (1)	AFLP	203 (1)
M11h03	CAC	TGT TGA ACC CTG ACC TGG TAG (for) ACC AGG TGA GCC AGC ATA GTC (rev)	50	100-103 (2)	88-109 (3)	NS	97 (1)	97 (1)	100 (1)	100-106 (2)	94-103 (3)	100-103 (2)	100-103 (2)	79-100 (3)	97-103 (3)	97-100 (2)
M14a11a	CAC	CCT CTG GAG GTA ACC TTG GTC (for) TCC ATG TTC ATG TTC GTG GTC (rev)	55	177	-	180-185 (2)	-	-	-	158 (1)	-	-	-	-	-	-
M11h07	CTT	GCT TCT TCT TCA TCT TCT TTA (for) AGT TCT CTT GGT CCT TTC TTA (rev)	55	220-226 (2)	211-220 (2)	186-229 (4)	196-226 (4)	-	214 (1)	-	-	200(1)	229(1)	223 (1)	AFLP-like	220 (1)
M11e11	AGA	AAA GGA GAA GAA TGA AAA TAA (for) TTT TAT CAT TCT ATC TTC CAT (rev)	55	180 183 (2)	180 195 (3)	180 198 (3)	180 206 (3)	-	180 (1)	180 (1)	180-199 (2)	204-211 (2)	175 (1)	204 (1)	264-292 (2)	-
M11g06	GT	TTT GTT GCA CGC AAA TGT CAC (for) CAG TGA TAA CAG TAC AAG AGG (rev)	55	111-113(2)	111 (1)	95-101 (3)	111-113 (2)	95 (1)	111 (1)	-	-	101-111 (2)	111 (1)	-	103 (1)	-
M11b05	GT	GGG TCT GTT TTA AGA AGA TAA AG (for) TAG ATT AAT GCC TTT AAA TGT AC (rev)	50	105 (1)	105 (1)	105 (1)	105 (1)	87 (1)	-	-	105 (1)	105 (1)	-	-	-	-
M11d04	ACC	TTT GAA TAG CGA TGA CGA TGA GC (for) GTT CTT CAT ACC CTT AAA GAT CG (rev)	50	103 (1)	103-106 (2)	120 (1)	103-109 (3) 371	159 (1)	100-115 (5)	103-106 (2)	-103 (1)	-103-112 (2)	-100-115(2)	103(1)	-	115(1)
M11d04_372	ACC			369-372	372 (1)	-	372	-	344 (1)	-	-	-	-	318 (1)	301 (1)	363 (1)

				(2)												
M20a03	GAA	TCA CGG GAA GTT TAC AAG TTG AAA G (for) GAA AAG GGA ATG GAA GAT GAA AGA G (rev)	55	195-249 (4)	195-219 (4)	230-239 (4)	228-243 (3)	-	195-216 (3)	195-210 (2)	195-210 (2)	195-210 (2)	195-210 (2)	195-210 (2)	165-219 (3)	165-225 (6)

bp, base pairs

Table 3. Characteristics of new *Tetranychus urticae* microsatellite loci in cross amplification in Phytoseiidae species. Allele size range (in base pairs) and number of alleles (within brackets) or amplification pattern are indicated.

Locus	Nc	Es	Pp	As	Aa	Ac	Tp	Nb
M11b04	70-82 (4)	70-85 (4)	70-76 (3)	70-82 (4)	70-85 (5)	52-64 (5)	52-64 (5)	52-64 (5)
M11g09	AFLP	146-152 (2)	AFLP	AFLP	221 (1)	AFLP	AFLP	AFLP
M11e03	AFLP	AFLP	AFLP	AFLP	AFLP	AFLP	AFLP	AFLP
M11h03	88-114 (5)	94-103 (3)	100-117 (2)	100-103 (2)	82-117 (5)	94-103 (3)	97-103 (3)	82-103 (4)
M14a11a	-	-	-	-	-	-	-	-
M11h07	220 (1)	-	220 (1)	221-202 (2)	220 (1)	-	208 (1)	208 (1)
M11e11	217-304 (3)	171351 (3)	239 (1)	-	221 (1)	-	183	180
M11g06	-	105-117 (2)	138 (1)	102-138 (3)	105-126 (3)	123-138 (2)	111-138 (2)	111 (1)
M11b05	105 (1)	105 (1)	-	-	105 (1)	105 (1)	105 (1)	105 (1)
M11d04	335-360 (2)	321 (1)	354 (1)	327 (1)	351 (1)	102 (1)	102-105 (2)	102-118 (2)
M20a03	192- 222 (4)	165-222 (5)	165-222 (6)	168-219 (6)	165-219 (6)	228 (1)	228-231 (2)	228 (1)

AFLP, amplification pattern as AFLP-like; -, no amplification

Table 4. Loci discarded by amplification failure or multiple banding results (AFLP-like loci) in *Tetranychus urticae* samples

Locus	Motif	Primer sequences (5'→3')	Size (bp)*	Annealing T (ºC)	Amplification type	Species tested
TuCA1	GT	CGA ATC ATA AAG AGA ATG GAG (for) TTC ATC TGG CTA TCT GGT GTC (rev)	373	55	AFLP	TuCS
TuGTG1	CAC	GAG CCT GAG ATT GAC GAT GAG (for) TCA GCA TCA CAA TCA GAC TCC (rev)	124	55	AFLP	TuCS
TuGT1	GT	TGG GAA GAT GAT GGT TTA ATG A (for) TTG CAT GCT TAA GGC CAT TT (rev)	200	55	AFLP	TuCS
M16f04	CAC	ATC GCT GGT GGA AAC AAA AGC (for) ATC ACT GTC CAC TAT CGT CAC (rev)	122	55	AFLP	TuCS
M11b12	CTT	AAA ATG TCA GTC AGT CTC AAT (for) GTA AAG GAA AAA TCT CAA AAA (rev)	191	55	AFLP	TuCS
M14g11	CAC	GAG TTT ATT TGA TGT TGA GGA CGA T (for) TTT TTG GGT CTT CGC TGG GGT AAC T (rev)	148	55	AFLP	TuCS
M17d08	GTG	AAG CGC AAC TAG ATT GAC GTT GAT G (for) ACG ATG AGT CCT GAG TAA TAA TGG G (rev)	115	55	No amplification	TuCS
M14e02	CTT	ACG ATG AGT CCT GAG TAA GTG (for) GAT TAT TTT TGC TTG GGA AGC (rev)	132	55	AFLP	TuCS
M14d02	CTT	TCC TCA TCA TCA TCA TCT TCT (for) ATC TTT ATT CCC TTT ATT TCA (rev)	125	55	AFLP-No amplification	TuCS
M11c09	CTT	AAT GAA AGA AGT TGA AAG TTG CT (for) CCA ATC CAA TGA ATA ACA TTG AG (rev)	318	50	No amplification	TuCS

*, expected size based on sequence data

Table 5. Summary of cross-species amplification of *T. urticae* microsatellite markers in 17 mite species.

Family	**Specie**	**Functional primer pairs**
Tetranychidae	*Tetranychus urticae* *	11/22
	T. turkestani	10/22
	T. evansi	8/22
	T. okinawanus	8/22
	Tetranychus	7/11
	Eotetranychus orientalis	8/22
	E. banksi	9/22
	Eotetranychus	7/9
	Panonychus citri	8/22
	Oligonychus perseae	8/22
	Aplonobia histricina	7/22
Phytoseidae	*Typhlodromus phialatus*	8/22
	Neoseiulus californicus	7/22
	N. barkeri	8/22
	Neoseiulus	7/8
	Euseius stipulatus	7/22
	Phytoseiulus persimilis	7/22
	Amblyseius swirskii	6/22
	A. andersoni	9/22
	A. cucumeris	6/22
	Amblyseius	5/9

The functional primer pairs number corresponds to those primer pairs that successfully amplified; *: mean of the five populations tested.