Recent emergence and worldwide spread of the red tomato spider mite, [i]Tetranychus evansi[/i]: genetic variation and multiple cryptic invasions

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ORIGINAL PAPER

Recent emergence and worldwide spread of the red tomato spider mite, *Tetranychus evansi*: genetic variation and multiple cryptic invasions

Recent emergence and multiple cryptic invasions of *Tetranychus evansi*

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Abstract Plant biosecurity is increasingly challenged by emerging crop pests. The spider mite *Tetranychus evansi* has recently emerged as a new threat to solanaceous crops in Africa and the Mediterranean basin, with invasions characterized by a high reproductive output and an ability to withstand a wide range of temperatures. Mitochondrial (868 bp of *COI*) and nuclear (1,137 bp of ITS) loci were analyzed in *T. evansi* samples spanning the current geographical distribution to study the earliest stages of the invasive process. The two sets of markers separate the samples into two main clades that are only present together in South America and Southern Europe. The highest *COI* diversity was found in South America, consistent with the hypothesis of a South American origin of *T. evansi*. Among the invaded areas, the Mediterranean region displayed a high level of genetic diversity similar to that present in South America, that is likely the result of multiple colonization events. The invasions of Africa and Asia by *T. evansi* are characterized by a low genetic variation associated with distinct introductions. Genetic data demonstrate two different patterns of invasions: (1) populations in the Mediterranean basin that are a result of multiple cryptic introductions and (2) emerging invasions of Africa and Asia, each likely the result of propagules from one or limited sources. The recent invasions of *T. evansi* illustrate not only the importance of human activities in the spread of agricultural pests, but also the limits of international quarantine procedures, particularly for cryptic invasions.

Keywords *Tetranychus evansi* · Mitochondrial DNA · Emerging pest · Multiple introductions · Cryptic invasions

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Introduction

The increase in movement of both people and commodities internationally favours the spread of organisms outside their indigenous ranges. In the newly colonized areas, exotic species often become pests with dramatic consequences for biodiversity, agriculture, and human health. A recent survey of the causes of new plant disease emergence worldwide concluded that 56% of recent outbreaks result from

introductions associated with trade of plants and plant products and the movement of people (Anderson et al. 2004). In the case of invasive arthropods, annual damages caused by exotic insects and mites in the United States alone have been estimated to be at least $16 billion (Pimentel et al. 2005). Climate change is likely to complicate further the impact of invasive plant pests. For example, a lengthening of the growing season in mid- and high latitudes of the northern hemisphere has been reported as a clear indicator of the responses by insect pests to a warmer climate (Menzel et al. 2008). As a result of both increased trade and climate change, emerging crop pests are likely to present new concerns for plant biosecurity, motivating research and new technologies to detect, identify and monitor pests and infectious diseases, and to modify strategies for plant crop protection (Rodoni 2009; Waage and Mumford 2008).

The red tomato spider mite *Tetranychus evansi* likely originated in South America and has emerged in only the last 10 years as a new destructive pest of solanaceous crops in many parts of the world. First reported from North-East Brazil in 1952 (Silva 1954), the mite was not considered a harmful pest in South America, except for a few outbreaks discovered in limited areas of Brazil (Humber et al. 1981). In the past half century the mite has since been collected from other continents including North America, many countries in sub-Saharan Africa, some Indian and Atlantic Ocean Islands, the Mediterranean basin, as well as from several parts in the Pacific Ocean (Hawaii, Taiwan, Japan and the south-eastern coast of China; see Migeon and Dorkeld 2006; Migeon et al. 2009 for a complete and chronological list). In the past decade, the pest status of *T. evansi* has changed and it is now regarded as a harmful invasive species in several parts in Africa and in the Mediterranean basin. In Africa *T. evansi* has become one of the most important dry season pests of tomatoes, causing yield losses of up to 90% in South-East Africa (Sibanda et al. 2000) and West Africa (Duverney and Ngueye-Ndiaye 2005). In the Mediterranean region, where several solanaceous field and glasshouse crops are economically important (tomato, potato, eggplant, etc.), the high invasive potential of *T. evansi* has prompted the recent addition of the species to the European and Mediterranean Plant Protection Organization (EPPO) alert list (EPPO 2007).

Invasion of new areas by *T. evansi* is favored by its high intrinsic rate of increase within a broad range of temperatures (Bonato 1999). With an arrhenotokous mode of reproduction (females being diploid and haploid males developing from unfertilized eggs), a small number of founding individuals or even a single female can initiate new mite colonies that can build up rapidly as a result of several typical 'r-selected' traits including short generation time (Sabelis 1985) and high dispersal ability (Kennedy and Smitley 1985). In addition, because of its small size (0.3–0.5 mm depending on the development stage), *T. evansi* can be difficult to detect on plant shipments and may remain undetected in new localities until its presence is revealed by outbreaks and plant damage. These factors, often compounded by the fact that historical records for the species are incomplete, inaccurate or nonexistent, make it difficult to reconstruct reliably the invasion history. Information on population histories gained by genetic approaches is particularly valuable for species such as *T. evansi*, that are small or inconspicuous (Navia et al. 2005; Scheffer and Grissell 2003) and that resemble morphologically resident species or previous invaders (Müller 2001; Reitzel et al. 2008; Stepien and Tumeo 2006). Such undetected cryptic invasions are not uncommon for spider mites, eventually leading to considerable economic consequences (e.g. Knapp et al. 2003; Navajas et al. 2001).

This paper presents a genetic analysis of the emerging pest *T. evansi,* using two DNA sequence based markers, a fragment of the Cytochrome Oxidase subunit I (*COI*) of mitochondrial DNA (mtDNA) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (rDNA). We include samples from the known geographical distribution of the species in South America, Africa, Asia and Southern Europe. Special effort in sampling the Mediterranean basin was made to understand the invasion process in this area, where *T. evansi* is currently rising as a significant pest. As a result of the coupling of: (a) information about the complex patterns of movement and historical demography obtained by genetic analysis, (b) knowledge of biological features associated with rapid population growth, and (c) the existence of reference collections that allow the predictions of potential distributions in the face of climate change (see Migeon et al. 2009), studies of *T. evansi* can be a model for understanding invasions of cryptic emerging invasive species.

Materials and methods

Sample collection

This study includes specimens of *T. evansi* from most parts of the world where it is currently known, including South America (Brazil and Argentina)—the assumed native area of the species—invaded areas on three continents (Africa, Europe and East Asia), where *T. evansi* has previously been reported, as well as additional new records (supplementary Table 1). Mites were collected between 2004 and 2008 from plants in the family Solanaceae and stored in 95% ethanol at −20°C for DNA analysis. Some individuals from each sample were preserved in 70% ethanol for further morphological verification of species identity involving observations of the shape of the male aedeagus under microscope preparations.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated from individual adult females using the DNeasy tissue Kit (Qiagen, USA), following the protocol described in (Tsagkarakou et al. 2007a). Two target DNA fragments were PCR-amplified and sequenced: a fragment of the *COI* mitochondrial gene (*COI*) and the internal transcribed spacer region (ITS: ITS1-5.8sRNA-ITS2). Several mites (1–9) per locality were sequenced for the *COI* (supplementary Table 1). Given the striking lack of variation of the ITS sequences with only two majority types detected worldwide (see results below), only 1–2 mites per locality were sequenced for this fragment. PCR conditions were as described previously (Tsagkarakou et al. 2007a) with modifications of annealing temperatures and primers used to amplify and sequence the ITS 1,137 base pairs (bp) and the *COI* 868 (bp) as described by Gotoh et al. (2009). PCR amplifications were carried out in an Eppendorf Mastercycler. PCR products were commercially sequenced (either by Cogenics, Meylan, France or Macrogen, Seoul, South Korea). GenBank accession numbers are included in Table 1.

DNA sequences alignment and analyses

Sequences were edited in the Seqscape package (SeqScape Software v.2.5, Applied Biosystems SeqScape® Software), and aligned using the default parameters of CLUSTAL W (Thompson et al. 1994) and refined by eye. There were no insertions/

Table 1 Variable nucleotide sites in mitochondrial *COI* (a) and ITS ribosomal (b) sequences resulting in ten *COI* haplotypes (H1–H10) and three ITS types (T1–T3) and separated in two major clades, I and II (see Fig. 2)

a

Clade	*COI* haplotypes	Variable sites	Nucleotide divergence %										Genbank accession numbers
		1111122 2223344455 566777778 1160001611 7993713625 833015574 0372398027 7055920939 147353784	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	
I	H1	CTCAATGCAT TAACGCTCTG AGGTAGCAT	-										FJ440678
I	H2	....T....	0.12	-									GU145106
I	H3	....A..C...	0.23	0.35	-								GU145107
I	H4	.....C	0.12	0.23	0.35	-							FJ440677
I	H5	....T..	0.12	0.23	0.35	0.23	-						GU145108
I	H6	.... G.....	0.12	0.23	0.35	0.23	0.23	-					GU145109
II	H7	TCTGGAATG. CCG.A..TCA .AACTATGC	**2.84**	**2.96**	**3.08**	**2.96**	**2.71**	**2.96**	-				FJ440676
II	H8	TCTGGA.TG. CCG.A...CA .AACTA..C	**2.35**	**2.47**	**2.59**	**2.47**	**2.47**	**2.47**	0.46	-			FJ440675
II	H9	TCTGGAATG. CCG.A.C.CA .AACTA..C	**2.59**	**2.71**	**2.59**	**2.71**	**2.71**	**2.71**	0.46	0.23	-		GU145110
II	H10	TCTGGAATG. CCG.A..TCA .AACTA.GC	**2.71**	**2.84**	**2.95**	**2.84**	**2.84**	**2.84**	0.12	0.35	0.35	-	GU145111

b

	ITS types	11 2700 8244 7358	T1	T3	T1/T3	T2	
I	T1	GAAA	-				FJ440674
I	T3	...G	0	-			GU145105
I	T1/T3	R..R	0	0	-		
II	T2	.GG.	**0.18**	**0.18**	**0.18**	-	FJ440673
II	T1/T2	.RR.					

Pairwise percent divergence for *COI* haplotypes and ITS types belonging to the two majors clades were calculated (interclade divergences in bold). Positions of variable sites listed vertically above each site correspond to sequences deposited in Genbank. Dots indicate sequence matches to the first sequence

deletions in either *COI* or ITS regions. The boundaries of genes in the ITS regions were delimited by comparison to the ITS sequences in Hurtado et al. (2008), GenBank accession no. AM408033. Pairwise nucleotide divergence of *COI* and ITS sequences using the Kimura 2-parameter (K2P) model were calculated using the program Molecular Evolutionary Genetic Analysis (MEGA v.4, Tamura et al. 2007) and phylogenetic trees were constructed using the neighbour joining method (Saitou and Nei 1987). For comparison, we also generated a maximum likelihood tree [implemented in PhyML v.2. 4.4 (Guindon and Gascuel 2003)] employing the GTR + I model, as determined by Modeltest v.3.7 (Posada and Crandall 1998). The *COI* sequence of the closely related *Tetranychus urticae* (obtained in this study) was used as outgroup. Samples were pooled by country to calculate haplotype frequencies (supplementary Tables 1 and 2). When haplotype diversity within a country was high (i.e., presence of two haplotype clades, see below), samples were divided into regions (i.e., within Brazil, France, Spain and Portugal). In some cases, localities of several countries were pooled by geographical affinities based on sample size and sequence homogeneity (i.e., Kenya and Tanzania were pooled in the East Africa sample and the Argentinean sample from Corrientes was included in the Brazil-South West sample).

Indices of sequence diversity, including number of mtDNA haplotypes (*Nh*), haplotype diversity (*h*), nucleotide diversity (π) and average number of pairwise nucleotide differences (*K*), were estimated using DnaSP v.4.20.2 (Rozas et al. 2003). These parameters were calculated for samples grouped into four geographical regions: (1) South America; (2) Mediterranean region (including Portugal, the Canary and the Madeira Islands); (3) Africa (including also Reunion Island), and (4) Asia; as well as for the two detected clades. Molecular analysis of variance (AMOVA; Excoffier et al. 1992) as implemented in Arlequin v.3.1 (Excoffier et al. 2006) was used to assess the genetic differentiation among the four geographical regions (South American, Mediterranean region, Africa and Asia). Genealogical relationships among haplotypes of *COI* were reconstructed by a haplotype network analysis obtained by statistical parsimony method (default parsimony connection limit of 95%) implemented in the TCS v.1.21 software (Clement et al. 2000).

Results

Mitochondrial *COI* haplotyes

Analysis of 868 (bp) of mitochondrial *COI* DNA sequence data from 298 individuals revealed ten unique haplotypes. Among the sequences analysed, 29 segregating (polymorphic) sites were detected (Table 1a), 27 of which were parsimony-informative and two were singleton sites. Ten different haplotypes were identified representing 0.697 ± 0.021 and 0.0123 ± 0.0053 (mean $\pm$ SD) haplotype and nucleotide diversity, respectively.

The distribution of the *T. evansi* mtDNA haplotypes across the sampled continents is presented in Fig. 1. Several haplotypes were found in a single locality (H2, H3, H5, H6, H8, H9 and H10) while others were widely distributed (H1, H4 and H7). The most widely distributed haplotype (H1) was found with a global frequency of 48.7% in Africa, Europe and South America (Brazil-South West) (supplementary Table 2; Fig. 1). The two other most frequent haplotypes were H4 and H7 with global frequencies of 16.1 and 17.1%, respectively. Whereas H4 occurred widely (Africa, Asia and Europe), H7 was found in only three localities in Europe (France-South, Spain-North East, and Portugal). In most localities a single haplotype was detected, but in others either two (Brazil-South West, Portugal Center, and Reunion Is.) or three (Spain-East) haplotypes were found (supplementary Tables 1 and 2).

The highest *COI* diversity was found in South America with six out of ten haplotypes present ($h = 0.685 \pm 0.031$, $\pi = 0.0137 \pm 0.0004$, $K = 11.8$) (Table 2). Among the invaded areas, the Mediterranean region where four haplotypes were detected, displayed the highest genetic diversity ($h = 0.642 \pm 0.020$, $\pi = 0.0122 \pm 0.0008$, $K = 10.6$), which was similar to the level of genetic diversity in South American samples (Table 2a).

The phylogenetic reconstruction based on *COI* sequences separates the samples into two clades (99% of support through bootstrap analysis, Fig. 2a). The maximum likelihood tree (not shown) generated using GTR+I model was not different from the NJ topology shown and also supported the two major clades with high bootstrap values (Fig. 2a). Haplotypes in each clade were closely related and separated from each other by only one, and in a few

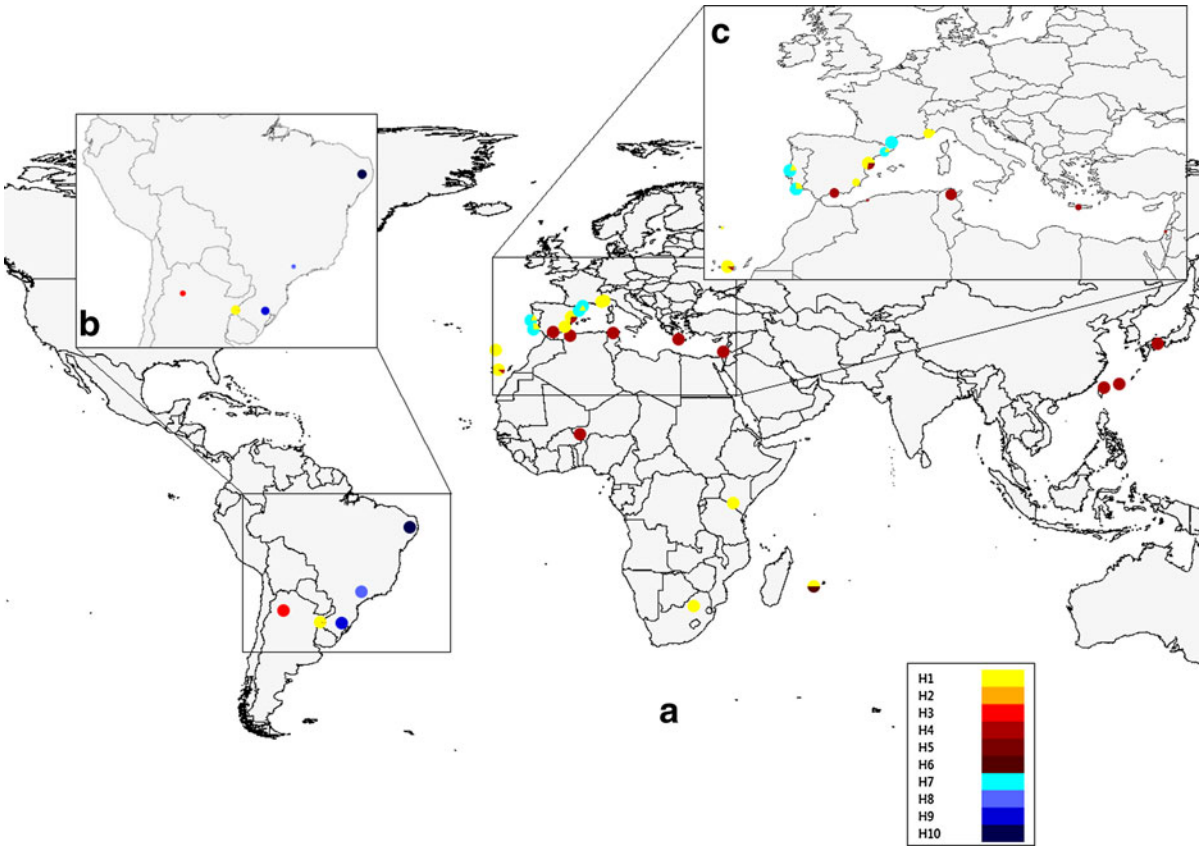

Fig. 1 **a** Distribution of mitochondrial DNA haplotypes of *Tetranychus evansi* across the geographical areas analyzed in this study. Nearby populations are pooled with the size of the symbols proportional to the number of individuals sampled: **b** in South America and **c** in the Mediterranean Basin. Pie charts illustrate the proportion haplotypes at each sampled locality

Table 2 Number of analysed individuals (*n*), polymorphic sites (*S*), parsimony informative sites (*PIS*), number of halplotypes (*Nh*), haplotype diversity (*h*), nucleotide diversity (π) and average number of differences (*K*) in mtDNA sequences computed for *Tetranychus evansi* mites collected from four geographical regions (a) and for two major clades (I and II; b)

	n	*S*	PIS	*Nh*	*h*	π	*K*
(a)							
South-America	78	26	25	6	0.6850 ± 0.0310	0.0137 ± 0.0004	11.8
Mediterranean region	166	25	25	4	0.6420 ± 0.0200	0.0122 ± 0.0008	10.6
Africa	42	2	1	3	0.2510 ± 0.0810	0.0003 ± 0.0001	0.3
Asia	12	0	0	1	0	0	0
(b)							
Clade I	203	6	4	6	0.4350 ± 0.0330	0.0006 ± 0.0001	0.5
Clade II	95	5	5	4	0.5950 ± 0.0300	0.0014 ± 0.0002	1.2

cases two, nucleotide difference/s. Clade I showed a lower diversity ($h = 0.435 \pm 0.033$, $\pi = 0.0006 \pm 0.0001$, $K = 0.5$) than Clade II ($h = 0.595 \pm 0.030$, $\pi = 0.0014 \pm 0.0002$, $K = 1.2$ Table 2b), included the two most common haplotypes worldwide (H1 and H4) and was represented in samples from all

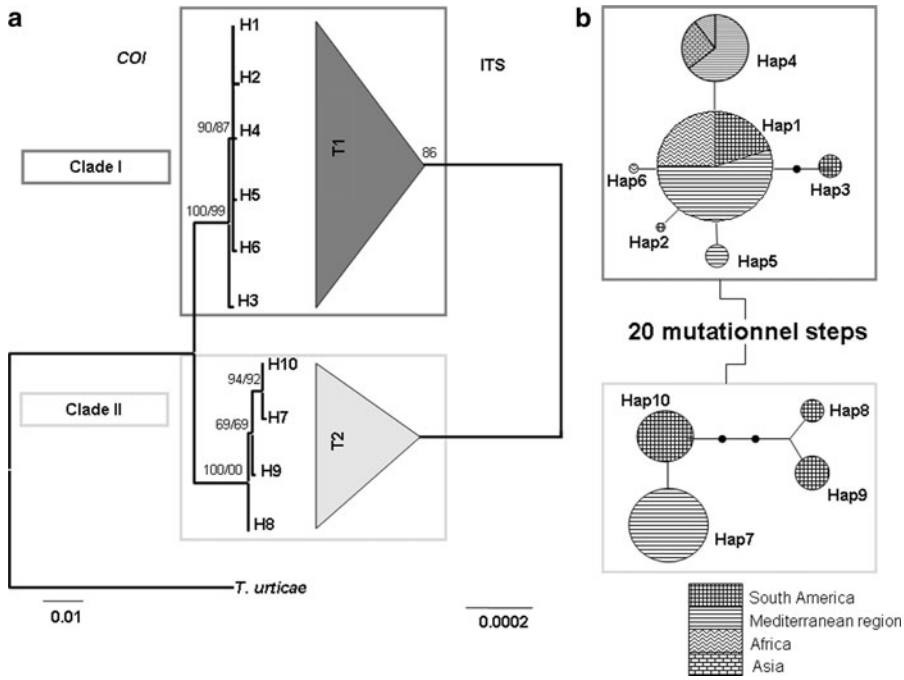

Fig. 2 **a** Phylogenetic reconstruction inferred through Neighbor-Joining based on mitochondrial *COI* (*left*) and ribosomal ITS sequences (*right*) from individual *Tetranychus evansi*. *Numbers* on branches indicate in the order bootstrap values for Neighbor-Joining and Maximum likelihood trees. *Tetranychus urticae* was used as outgroup. **b** A mitochondrial haplotype network constructed from samples of *T. evansi* using statistical parsimony. The areas of the *circles* are proportional to the number of samples sharing each haplotype. *Lines* represent a single mutational step and small *black circles* represent an unobserved haplotype

continents. Clade II was represented in South American and Mediterranean samples only. These patterns of geographical differentiation support those from the TCS network, in which the two clades are separated from each other by 20 mutational steps (Fig. 2b). *COI* sequence divergence between the two clades averaged 2.72% in contrast to intra-clade divergences, which ranged from 0.12 to 0.35% and from 0.12 to 0.46% for clades I and II, respectively (Table 1a). Analysis by AMOVA shows that the genetic differentiation within each geographical region explains most (79%) of the total variance (Table 3), which results from the presence of sequences of the two clades in South America and the Mediterranean region.

Internal Transcribed Spacer (rDNA ITS) variation

The complete ITS region (1,137 bp) was sequenced for a total of 96 individuals. Two major types of ITS sequences were found, named here T1 and T2, which differed from each other by two point mutations in the ITS2 region (positions 723 and 1,045). Variation among the two ITS types were consistent with the

Table 3 Hierarchical analysis of molecular variance (AMOVA) and estimators of genetic differentiation calculated for samples of *Tetranychus evansi*

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	*F*st
Among localities	3	228.327	1.26450 Va	20.97	0.20970*
Within localities	287	1368.920	4.76976 Vb	79.03	
Total	290	1597.247	6.03426		

Two sources of variation have been tested for four geographical regions (South America, Mediterranean region, Africa and Asia)

* represents $P < 0.01$, 1000 permutations

two *COI* clades revealed by the mtDNA sequence data. The single exception was ITS sequences (T3) found in the Argentina sample, which differed from T1 by a single point mutation (Table 2b). Four heterozygotes (T1/T3) were detected in this Argentinean sample of five individuals. Otherwise, ITS heterozygotes were rare: one individual from Portugal Center was a T1/T2 heterozygote. The most frequent (70.83%) and widely distributed ITS type was T1, which was found in most of the invaded areas as well as South West Brazil and Argentina (supplementary Table 2). The T2 type (22.92%) was found in some parts of the West Mediterranean region (France-South, Spain-North East and Portugal) and in the Atlantic Brazilian Coast which includes the North East, South East and South Brazil samples.

Discussion

Origins of invasive *Tetranychus evansi*

A common assumption, though not always correct, is that the geographical natal range of a species contains the oldest populations which will therefore have the greatest genetic diversity (Lozier et al. 2009; Nardi et al. 2005; Roderick 2004). In this study, patterns of genetic variation of *T. evansi* are consistent with the hypothesis of a South American origin of the species. Populations in South America were found to contain six out of the ten identified mitochondrial haplotypes and the single variant (T3) of the most common ITS sequence (T1), despite a relatively small sample size (the cumulative number of individuals analyzed in the other continents was approximately three times as great). Although the measured genetic diversity of *T. evansi* would certainly increase with a greater number of populations examined (as modeled for several invasive species, Puillandre et al. 2008), the sample from South America comprises collections from Recife (Brazil) to Tucuman (Northern Argentina), covering more than 3,800 km, spanning the distribution area of the pest. *Tetranychus evansi* is not evenly distributed in Brazil and geographical predictions based on climatic modelling pinpointed the Atlantic coast and South Brazil as the most suitable geographical areas for the species (Migeon et al. 2009). More generally, based on predictions modelled by CLIMEX by these authors, the potential distribution of *T. evansi* worldwide appears limited by cold stress together with the impact of other climatic parameters, mainly dryness and humidity. The restricted geographical distribution of *T. evansi* in South America, which has been confirmed by several intensive surveys (Da Silva et al. 2008; Furtado et al. 2005; Furtado et al. 2006) might be explained by climatic conditions, but could also reflect other environmental or ecological factors. Low densities of *T. evansi* were generally observed in South America compared to most of the newly invaded continents where the mite undergoes heavy outbreaks, such as in Africa (Saunyama and Knapp 2003) and in Southern Europe along the Spanish Mediterranean coast (Ferragut and Escudero 1999).

Among the factors that might account for different mite densities in the native and invaded areas is competition with other tetranychid species, which are abundant on solanaceous plants in Brazil [32 and 17 tetranychid species have been reported from solanaceous plants in South America and Europe, respectively (Migeon and Dorkeld 2006)]. In addition, effective native natural enemies in South America maintain *T. evansi* populations at low densities (Furtado et al. 2005; Furtado et al. 2006; Rosa et al. 2005). For example the neotropical predatory mite *Phytoseiulus longipes* has been described as successfully developing on *T. evansi* (Maxime Ferrero, personal communication; Furtado et al. 2007), and several other mite predators of the family Phytoseiidae have been reported associated with *T. evansi* on tomato and wild solanaceous plants in Brazil (Fiaboe et al. 2006; Furtado et al. 2006; Rosa et al. 2005). A fungal pathogen was also suggested as the causative agent of the decline of *T. evansi* populations in northeastern Brazil, when in 1979 occasional outbreaks in tomato field crops were reported (Humber et al. 1981). However, outside of South America, several attempts to control *T. evansi* with different mite predators that have been used successfully on other *Tetranychus* species, have not shown promising results (Escudero and Ferragut 2005; De Moraes and McMurtry 1985). These findings are consistent with the enemy release hypothesis, which predicts less predatory pressure in introduced populations (Keane and Crawley 2002). Knowledge of a South American origin of *T. evansi* should help to target regions for additional exploration of biocontrol candidates (see Roderick and Navajas 2003).

Species-wide genetic homogeneity and invasion events

Assuming a South American origin of *T. evansi,* the invasion of new areas by this mite sampled in this study, except Mediterranean region, was characterized either by a very low genetic diversity (e.g., Africa) or no genetic variation (e.g., East Asia). A paradox often encountered in genetic studies of invasive species, is a colonization success of the introduced populations despite a strong reduction of genetic diversity (Frankham 2005; see also reviews by Dlugosch and Parker 2008 and Roman and Darling 2007). Several studies to date have shown that founder effects and bottlenecks are not an obstacle for invasion success (Ahern et al. 2009; Puillandre et al. 2008; Solignac et al. 2005), whereas plasticity in life history traits seems to be important for the successful expansion of an invasive species (Chen et al. 2006; Valiente et al. 2010; Wang et al. 2005). Mites belonging to clade I have been found across a wide range of habitats and regions beyond the predicted geographical distribution as modeled based on climatic suitability *T. evansi* (Migeon et al. 2009). Although well established in the Mediterranean basin where climatic conditions are favorable to the species, haplotype H4 also occurs beyond the modeled climatic borders for the species, such as near the Southern Sahelian border, where according to modeling predictions (Migeon et al. 2009) dryness should limits the distribution. Likewise, in Asia the same haplotype was detected in areas predicted to experience stress through humidity (in Taiwan) or cold (in Japan). With an unexpected wide range of climatic conditions being tolerated by one of the *T. evansi* haplotypes, its increasing distribution is not likely to be well predicted by existing climate-based modeling, and special attention should be given to its expansion worldwide.

Colonization from multiple sources may mitigate the loss of genetic diversity needed to face novel selective challenges as encountered in the new invaded areas (Facon et al. 2006; Kolbe et al. 2004; Roman 2006). The genetic variability of mites from the western Mediterranean region was the highest among the colonized areas. Given that records of *T. evansi* in the colonized areas date from less than 50 years ago, and considering a mutation rate of mitochondrial genes typically estimated at 1.4–2.3% per million years in Arthropods (Xu et al. 2006), it seems unlikely that the haplotype diversity originated in the new region, but rather represents the combined effects of ancestral diversity and multiple introduction events. Although the level of divergence (ranging from 2.35 to 3.08%), between the two mtDNA clades detected might suggest the presence of two distinct taxa within a species complex, evidence of a single species exists. First, a comparable amount of intraspecific genetic diversity of *COI* sequences has been found within other Tetranychidae mite species (ranging from 1 to 6%) (Navajas and Boursot 2003; Navajas et al. 1994; Navajas et al. 1999; Navajas et al. 1998). Second, we have detected ITS sequence hybrids (each of the ITS types corresponds to one of the two *COI* clades) in the same sample (from Portugal), thus showing that crosses between mites stemming from the two clades are not only possible, but exist in nature. Additional evidence of conspecificity for individuals of the two clades comes from cross breeding experiments, which demonstrated fertile F1 females, although with some incompatibility (Gotoh et al. 2009).

Invasion pathways

In Africa, where the oldest records of *T. evansi* outside the Americas have been reported [in 1952 from the Mauritius Is. (Moutia 1958) and in 1979 from Zimbabwe (Blair 1983)], the colonization of wide areas by *T. evansi* results from unique introductions (for example, a single haplotype, H1, was detected in 13 samples from Kenya, Tanzania and South Africa). With H1 also encountered in Southwest Brazil, this region might be a potential source of the invasion of the African continent. Haplotype H4 from clade I, found in the Maghreb countries and Niger, is largely distributed in the Mediterranean basin and is the only one found in Japan and Taiwan. The virtual lack of genetic diversity in Asian samples supports a single and recent introduction event. The first documented record in Asia is from Taiwan in 2001 (Ho et al. 2004), although older reports of misidentified *T. evansi* from Taiwan exist back to 1992 (Ho and Wang 2007) with subsequent reports from Japan in 2002. All current mite records of *T. evansi* in Asian continent were from regions surrounding port cities such as Kagoshima, Tokyo, Osaka, Kyoto, Fukuoka and Okinawa in Japan (T. Gotoh, unpublished data) and from Taitung in Taiwan, suggesting the central role of human

commodities in the invasion of Asia by *T. evansi*. It remains however, unclear whether a single introduction event or several from the same source are responsible for colonization of this region. It cannot be ruled out that the Mediterranean region or the Western Africa, where mites bear the same haplotype, might have played a role as a secondary source in the colonization of *T. evansi* of the Asian continent.

Emerging pest: lessons learned by *Tetranychus evansi*

Among the different haplotypes present in the Mediterranean region, H1 and H4, both from clade I, are the most widely spread, whereas a single haplotype of clade II (H7) was found in a restricted area of the Mediterranean region. Worth noting is that H1 and H4 are in areas where heavy mite outbreaks have been reported, e.g. in South-East Africa (Saunyama and Knapp 2003; Sibanda et al. 2000), West Africa (Duverney and Ngueye-Ndiaye 2005), Spain (Ferragut and Escudero 1999), and where we have also observed the mite in the field on tomato and eggplant greenhouses in France and in the Canary Is. and on pepper and potatoes in Algeria (Yamina Guenaoui, personal communication). By contrast, high mite densities in regions where H7 and other haplotypes belonging to the clade II are present have never been reported and mite populations have been observed associated with wild solanaceous plants such as nightshade, *Solanum nigrum.* In addition to differences in host associations, the two clades also appear to differ in invasive potential, with mites from clade I having a higher invasive potential having colonized the entire Mediterranean basin, together with Africa and East Asia. Demographic parameters and adaptive performance underlying the differential invasive success of mites of clade I and II, are currently under investigation. The existence of two biologically distinct clades that differ in invasive potential is similar to what is known about the recent invasive biology of the tropical whitefly with *Bemisia tabaci*, which there exist at least two worldwide invasive biotypes, B and Q, (Boykin et al. 2007). Biotype Q is dominant in the Mediterranean basin and shows performance differences related to extreme temperature (Bonato et al. 2007) and to insecticide tolerance (Horowitz et al. 2005; Tsagkarakou et al. 2007b).

The threat that *T. evansi* represents for local agriculture in the Mediterranean basin is increased by the sympatric presence of distantly related mites. In the case of multiple introductions which might have unequal potential for becoming invasive (Allendorf and Lundquist 2003; Jousson et al. 2000; Sakai et al. 2001), crosses among individuals from distant populations might generate novel genetic combinations leading to new traits which eventually results in increased invasiveness (Allendorf and Lundquist 2003; Ellstrand and Schierenbeck 2000; Facon et al. 2006; Lee 2002). Hybrid vigour has been invoked to explain why some introduced populations may overcome their parents' fitness in new environments (Facon et al. 2005) and the reproductive compatibility between mites from the two clades has recently been demonstrated (Gotoh et al. 2009).

Tetranychus evansi is regarded as an important agricultural pest in Southern Europe but also in South East Africa and the detection of different lineages with different invasive potential is crucial to help in designing pest management strategies involving quarantine measures and the search for biocontrol candidates. The assumption that a local adaptation between biological control agents and the invasive genotypes of a pest exists (see Hufbauer and Roderick 2005), implies a targeted search of natural enemies in the area of provenance of the particular invasive genotypes. Among several mite predators reported associated with *T. evansi* in Brazil, only Brazilian (Furtado et al. 2007) and Argentine (Maxime Ferrero, personal communication) *P. longipes* populations were the most promising to control *T. evansi* populations in introduced areas. By contrast, the Chilean (Maxime Ferrero, personal communication) and South African populations (De Moraes and McMurtry 1985) of this mite predator are inefficient to control *T. evansi*.

This study illustrates the extent to which molecular markers can aid in the study of an emerging pest in the earliest stages of the invasion process and thus help to address questions related to management and biosecurity. Unfortunately, regulation generally does not take into account the fact that some genotypes of an invasive species may differ in invasive potential (Allendorf and Lundquist 2003). Human activities have played a significant role in explaining the present distribution of *T. evansi*, and thus monitoring pathways will be critical for its control. The spread of

T. evansi throughout the world is testimony of the limits of international quarantine measures, particularly important in the case of inconspicuous pests, which are prone to cryptic invasions. Integrating international cooperation will be critical to identify sources and pathways of invasion as well as build and/or maintain resilience to invasion to enhance plant biosecurity for emerging arthropod pests.

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