10-2012

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Genetic Characterization of North American Populations of the Wheat Curl Mite and Dry Bulb Mite

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J. Econ. Entomol. 105(5): 1801–1808 (2012); DOI: http://dx.doi.org/10.1603/EC11428

ABSTRACT The wheat curl mite, *Aceria tosichella* Keifer, transmits at least three harmful viruses, wheat streak mosaic virus (WSMV), high plains virus (HPV), and Triticum mosaic virus (TriMV) to wheat (*Triticum aestivum* L.) throughout the Great Plains. This virus complex is considered to be the most serious disease of winter wheat in the western Great Plains. One component of managing this disease has been developing mite resistance in wheat; however, identification of mite biotypes has complicated deployment and stability of resistance. This biotypic variability in mites and differential virus transmission by different mite populations underscores the need to better understand mite identity. However, *A. tosichella* has a history of serious taxonomic confusion, especially as it relates to *A. tulipae* Keifer, the dry bulb mite. Molecular techniques were used to genetically characterize multiple *A. tosichella* populations and compare them to populations of *A. tulipae*. DNA from these populations was polymerase chain reaction amplified and the ribosomal ITS2 region sequenced and compared. These results indicated limited variability between these two species, but two distinct types within *A. tosichella* were found that corresponded to previous work with Australian mite populations. Further work using sequencing of several mitochondrial DNA genes also demonstrated two distinct types of *A. tosichella* populations. Furthermore, the separation between these two *A. tosichella* types is comparable to their separation with *A. tulipae*, suggesting that species scale differences exist between these two types of *A. tosichella*. These genetic differences correspond to important biological differences between the types (e.g., biotypic and virus transmission differences). In light of these differences, it is important that future studies on biological response differences account for these mite differences.

KEY WORDS *Aceria tosichella*, *Aceria tulipae*, wheat curl mite, wheat streak mosaic

Wheat streak mosaic has long been considered the most serious disease of winter wheat (*Triticum aestivum* L.) in the western Great Plains (Brakke 1987). However, the wheat curl mite, *Aceria tosichella* Keifer, has now been shown to transmit three serious viruses, wheat streak mosaic virus (WSMV), high plains virus (HPV), and Triticum mosaic virus (TriMV) to wheat throughout the Great Plains (Slykuis 1955; Seifers et al. 1997, 2008). HPV was identified from wheat and corn throughout the Great Plains in the mid-1990s (Jensen and Lane 1994, Jensen et al. 1996), and TriMV was recently identified from wheat in Kansas (Seifers et al. 2008). Because the wheat curl mite transmits all of these viruses, they are often found together in mixed infections in the field (Mahmood et al. 1998, Seifers et al. 2008). The extent of the interaction of the viruses is not known; however, it is clear that where one or more viruses are present, the resulting disease complex significantly impacts wheat (Tatineni et al. 2010, Byamukama et al. 2012).

As with most arthropod-transmitted pathogens, management of the virus complex focuses on managing the vector. The most prevalent problems with this disease complex arise when hail shells out seeds from headed wheat just before harvest, and the fallen seeds quickly germinate and produce volunteer wheat (Brakke 1987, Wegulo et al. 2008). This volunteer wheat is rapidly infested with mites moving from the maturing wheat and serves as a 'green bridge' to carry both the mite and viruses through the summer to infest and infect the newly emerging winter wheat in the fall. Cultural control practices are targeted at breaking the green bridge by eliminating volunteer wheat, and thus, reducing the potential for fall infections (Wegulo et al. 2008, Hein 2010). Often, environmental conditions make volunteer wheat control problematic, and the disease potential persists. In addition, other alternate summer hosts for the mites and viruses (e.g., corn, potentially several other grass hosts) can function as the green bridge to establish infections in the fall (Wegulo et al. 2008).

The significant impact of this disease in Great Plains winter wheat has resulted in a good deal of work being

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done to identify virus- and mite-resistant germplasm. The first wheat curl mite resistance gene that became widely used commercially originated from a translocation of rye into wheat and was deployed in the cultivar 'TAM 107' (Martin et al. 1984). TAM 107, and a few other varieties with the same gene, were planted widely in the central Great Plains in the late 1980s and 1990s, but in the mid-1990s, wheat curl mite populations in the region were found to have overcome this resistance gene (Harvey et al. 1995, 1997). Several additional genes for resistance to wheat curl mite colonization have been identified from several wheat relatives and transferred into wheat (Conner et al. 1991, Li et al. 2002).

A major drawback to widespread deployment of most of these resistant genes is that the reaction to these genes is inconsistent and dependent on the source of mites tested. Harvey et al. (1999) found a varied response to seven sources of resistance to wheat curl mite found in wheat when comparing five geographically distinct mite populations (Nebraska, South Dakota, Montana, Texas, and Alberta, Canada). They also found a varied response when comparing eight mite populations from across Kansas. Malik et al. (2003) also found biotypic differences between populations of wheat curl mite when comparing diverse germplasm. These differences between mite populations in response to resistance genes have serious implications to gene deployment and managing these genes to avoid further wheat curl mite biotype development.

Differences in response to mite resistance genes are not surprising in light of the historic record of serious taxonomic confusion for *A. tosichella* and the dry bulb mite, *Aceria tulipae* Keifer. Originally the wheat curl mite was misidentified as *A. tulipae* (Keifer 1954, Slykhuis 1955), but in 1970, Shevtchenko et al. (Shevtchenko et al. 1970) described *A. tritici* from wheat as distinct from *A. tulipae* on onions (Amrine 1996). However, Keifer just the year before had described *A. tosichella* from wheat in Yugoslavia (Keifer 1969), and thus, the name *A. tosichella* has priority over *A. tritici* for the wheat curl mite from wheat (Amrine and Stasny 1994). However, the correct name, *A. tosichella*, did not find its way into the literature after its original description, and generic name changes, where *A. tulipae* became *Eriophyes tulipae* (Newkirk and Keifer 1971), further confounded the issue. In 1994, Amrine and Stasny sorted out the original literature and descriptions and clearly separated the dry bulb mite, *A. tulipae*, from wheat curl mite, *A. tosichella*. However, questions still remained as to the identity of both mites as Frost and Ridland (1996) have indicated that both *A. tulipae* and *A. tosichella* are likely species complexes in Australia.

Molecular techniques have the potential to help resolve many of these difficult eriophyoid identification issues (Navajas and Navia 2010). Fenton et al. (1996) and later Kumar et al. (1999) and Lemmetty et al. (2001) used molecular techniques to delineate closely related *Cecidophyopsis* species (including *C. ribes*). Recently, several host-adapted strains of *Abacarus hystrix* (Nalepa) were identified using molecular genetics techniques and verified by using host and morphological data (Skoracka et al. 2002, Skoracka and Dabert 2010). Alternatively, examinations of genetic variation for *Aceria cajani* Channabasavanna from pigeonpea in India and the surrounding region demonstrated little variability (Kumar et al. 2001). For *A. tosichella*, recent molecular characterizations have shown haplotype differences in Australia (Carew et al. 2009).

More complete characterization of *A. tosichella* populations may enhance our understanding of the relationship of this mite to its hosts and also to the viruses that they transmit. Seifers et al. (2002), using mostly the same wheat curl mite populations that Harvey et al. (1999) used to demonstrate biotypic differences, demonstrated differential transmission of HPV by these various mite populations. They also found that these five populations of wheat curl mites all transmitted WSMV. However, Schiffer et al. (2009) identified two distinct strains of *A. tosichella* in Australia and found that they differ in their ability to transmit WSMV. Transmission of TriMV by wheat curl mite has been demonstrated (Seifers et al. 2009), but the influence of mite populations on transmission has not been fully investigated. However, preliminary data indicates that differential transmission by different *A. tosichella* populations may also occur (G.L.H., unpublished data). This variable capacity of mite populations to transmit HPV and perhaps TriMV has important implications on the epidemiology and management of this virus complex.

Because of the demonstrated importance of understanding the variability in mite populations, it is critically important to be able to distinguish between these different populations of mites. The capabilities of molecular genetics techniques may allow us to distinctly characterize the genetic identity of these populations of wheat curl mite from the Great Plains in North America and compare this mite species to its closely related species, the dry bulb mite, *A. tulipae.*

Materials and Methods

Five *A. tosichella* colonies originating from different states in the Great Plains (Kansas, Texas, South Dakota, Montana, and Nebraska) were used in this study. They were obtained from colonies that were established in 1999 from mites obtained from Drs. Thomas Harvey and Dallas Seifers, Kansas State University, Agricultural Research Center, Hays, KS. These were the same colonies that were used to test for biotypic differences by Harvey et al. (1999) and Malik et al. (2003) as well as demonstrate transmission differences between the state colonies for HPV (Seifers et al. 2002). In addition to these five *A. tosichella* colonies, another colony of *A. tosichella* was collected at the Panhandle Research and Extension Center in Scottsbluff, NE (labeled 'PREC'). Mites in these colonies were reared on mite susceptible wheat cultivars (cultivar 'Arapahoe' or 'Millennium') grown in caged pots and maintained in the greenhouse or growth cham-

Table 1. Primers used for ribosomal DNA and mitochondrial DNA PCR and sequencing reactions

Primer	Sequence	Reference
rDNA		
rDNA2	5′-TTGATTACGTCCCTGCCCTTT-3′	Cherry et al. (1997)
rDNA1.58S	5′-ACGAGCCGAGTGATCCACCG-3′	Cherry et al. (1997)
Mite A	5′-GTGAACCTGCGGAAGGATCA-3′	This article
Mite B	5′-ATTGGCTAGCAACCTAAGCA-3′	This article
Mite C	5′-TTGATTACGTCCCTGCCCT-3′	This article
mtDNA		
C1-J-2183	5′-AATCAAAAGTCTATTAATTGTAGACCTG-3′	Simon et al. (1994)
C2-N-3661	5′-CCACAAATTTCTGAACATTGACC-3′	Simon et al. (1994)
COIB	5′-GATACAGTTCTTCATGATACATATTAG-3′	This article
COIIA	5′-AGAAAGGAATAACTGTTCAAATTCTT-3′	This article
COIR	5′-ATAGAAATAGTAGCTAATCAACTAA-3′	This article
LR-N-12868	5′-TTACATGATCTGAGTTCAAACC-3′	Simon et al. (1994)
LR-J-12883	5′-CTCCGGTTTGAACTCAGATC-3′	Simon et al. (1994)
SR-N-14588	5′-AAACTAGGATTAGATACCCTATTAT-3′	Simon et al. (1994)
COIR	5′-ATAGAAATAGTAGCTAATCAACTAA-3′	This article
16SF	5′-AATCAAAAGTCTATTAATTGTAGACCTG-3′	This article

bers. To prevent cross contamination of the mites, pots for each individual caged colony were maintained either within separate cages or separate growth chambers. All colonies, except PREC, were maintained virus-free, and mites were regularly (ca. every 3–4 wk) transferred to new plant material. After these colonies were received in 1999, the number of mites in each colony was increased and large collections of mites from each colony were preserved in 100% ethanol and stored at −20°C for later assay. All mite colonies were identified by J.W.A. as *A. tosichella* through the use of morphological characteristics.

Two additional populations of *A. tosichella* were collected in Dundy Co., NE, in June 2005, Dundy1 and Dundy2. These mites were isolated from wheat heads, and colonies were initiated on Millennium wheat by using a single female transfer, resulting in a female clonal population that was allowed to increase before collection and storage in 100% ethanol.

The eriophyid mite, *Aceria kendalli* Baker, was collected from matrimony vine (*Lycium barbarum* L.) by J.W.A. near Morgantown, WV, to use as an outgroup in subsequent phylogenetic comparisons. Three *A. tulipae* populations, obtained from different sources, were used throughout the study. The Oregon source (OR) was obtained from garlic (*Allium sativum* L.) produced in Columbia Co., OR, in December 1997. A second source was obtained from a garlic producer in Leamington, Ontario, Canada (ON), in January 2000, and a third source (NE) was obtained from onions (*Allium cepa* L.) purchased in a local grocery store in western Nebraska (October 1999). All these mites were colonized on 'White Lisbon' onions for an extended period to build up mite numbers. Large numbers of mites were collected and preserved in 100% ethanol for later assay.

One to five mites were collected from infested plants or preserved samples and placed in 0.5 ml polymerase chain reaction (PCR) tubes in 20 μl 1× PCR buffer (Roche, Indianapolis, IN) and stored at −20°C before assay. Before PCR, mite-containing tubes were heated to 99°C for 5 min and then placed on ice. PCR primers (0.2–0.5 μM final concentration), deoxynucleotide triphosphates (final concentration of each 0.2 mM), 8 μl 10× PCR buffer, 2.5 U *Taq*DNA polymerase, and sufficient sterile water to make a final volume of 100 μl were added to each tube. To amplify the ribosomal internal transcribed spacer one (ITS1) region, primers rDNA2 and rDNA1.58S (Table 1) were used with 35 cycles of PCR steps of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. Following PCR amplification, products were purified using High-Pure spin columns (Roche) and stored at −20°C. The nucleotide sequence of both strands was obtained by automated sequencing (DNA Sequencing Facility, Iowa State University, Ames, IA) using primers rDNA1.58S, MiteA, MiteB, and MiteC (Table 1).

PCR primers C1-J-2183 and C2-N-3661 or COIB and COIIA (Table 1) were used to amplify partial mitochondrial DNA (mtDNA) sequences of cytochrome oxidase I (COI) and cytochrome oxidase II (COII) genes. Primers LR-N-12868 and LR-J-12883 were used in conjunction with COIR to establish the orientation of the 16S rRNA gene relative to the COI genes. Partial 12S and 16S gene sequences were amplified with primers SR-N-14588 and LR-J-12883. Finally, mtDNA spanning a region from the 16S to COI genes was amplified using primers 16SF and COIR (Table 1). DNA fragments were ligated to *t*-tailed pGEM-T Easy (Promega, Madison, WI) and transformed into *Escherichia coli* JM109. Plasmid DNAs were sequenced (Davis Sequencing, Inc., Davis, CA) by primer walking using a minimum of three independent clones per sequence. Automated sequence data were compiled using Sequencher 4.1 (Gene Codes, Ann Arbor, MI). Sequences of each region were aligned using CLUSTAL X (Thompson et al. 1997). Genetic distances were calculated for each sequence set using MEGA4 (Tamura et al. 2007). Phylogenetic analysis by maximum likelihood was done with the TREEFINDER computer program (Jobb et al. 2004) using the HKY (Hasegawa et al. 1985) model of nucleotide substitution. A search for potential tRNA genes was done with ARWEN (Laslett and Canbäck 2008).

Table 2. Pairwise percent nucleotide differences among ribosomal DNA ITS1 sequences for *A. tosichella* and *A. tulipae* populations

	A. tosichella								*A. tulipae* NE	*A. tulipae* OR	*A. tulipae* ON	*A. tulipae* CAN[a]
	NE	PREC	Dundy2	KS	MT	SD	TX	Dundy1				
A. tosichella PREC	0											
A. tosichella Dundy2	0	0										
A. tosichella KS	1.2	1.2	1.2									
A. tosichella MT	1.2	1.2	1.2	0								
A. tosichella SD	1.2	1.2	1.2	0	0							
A. tosichella TX	1.2	1.2	1.2	0	0	0						
A. tosichella Dundy1	1.2	1.2	1.2	0	0	0	0					
A. tulipae NE	1.5	1.5	1.5	0.3	0.3	0.3	0.3	0.3				
A. tulipae OR	1.5	1.5	1.5	0.3	0.3	0.3	0.3	0.3	0			
A. tulipae ON	1.5	1.5	1.5	0.3	0.3	0.3	0.3	0.3	0	0		
A. tulipae CAN[a]	1.2	1.2	1.5	0	0	0	0	0	0.3	0.3	0.3	
A. kendalli	37.8	37.8	37.8	36.6	36.6	36.6	36.6	36.6	36.9	36.9	36.9	36.6

[a] Genbank AJ251695 (probably wheat curl mite).

Results and Discussion

ITS1 sequences of other eriophyid mites have been useful in defining species relationships (Kumar et al. 1999). Therefore, this region was PCR amplified for eight regionally collected populations of *A. tosichella* (KS, MT, SD, TX, NE, PREC, Dundy1 and Dundy2), three populations of *A. tulipae* (NE, OR, and ON), and the outgroup, *A. kendalli* and then directly sequenced. The ITS1 region of the *A. tulipae* and *A. tosichella* populations was 398 nucleotides in length, except for *A. tulipae*-NE that was 397 nucleotides. The ITS1 region of *A. kendalli* was 330 nucleotides. Genetic distances are presented in Table 2. Over all, there were limited sequence differences among the *A. tulipae* and *A. tosichella* populations. The sequences from the three *A. tulipae* populations were identical to each other, except for the one nucleotide deletion for *A. tulipae*-NE. Similarly, the KS, MT, SD, TX, and Dundy1 *A. tosichella* populations had identical sequences. The *A. tosichella* PREC and NE populations were collected from the same area and had the same ITS1 sequences, as did the Dundy2 population. These three Nebraska populations of *A. tosichella* were 1.2% divergent from the KS, MT, SD, TX, and Dundy1 populations of *A. tosichella*. The three *A. tulipae* populations (NE, OR, and ON) were slightly more divergent from the NE, PREC, and Dundy 2 populations of *A. tosichella* (1.5%) than the KS, MT, SD, TX, and Dundy1 *A. tosichella* populations (0.3%). In contrast, *A. kendalli* was 36–38% different from all *A. tulipae* or *A. tosichella* populations tested.

Sequence divergence of all the *A. tosichella* and *A. tulipae* populations tested from the ITS1 of an additional outgroup, *A. pongamiae* (Kumar et al. 2001), was over 40%. One sequence of *A. tulipae* collected from wheat in Canada reported from GenBank (AJ251695; Kumar et al. 2001) showed slightly more divergence from the NE, PREC, and Dundy2 populations of *A. tosichella* reported here (1.2%) than from the three *A. tulipae* populations tested here (0.3). However, this GenBank sequence was identical to the KS, MT, SD, TX, and Dundy1 *A. tosichella* population sequences reported here (Table 2). This suggests that this population actually represents an *A. tosichella* population, and this would be suspected as it was a collection from wheat. This example underscores the considerable confusion between these species that has occurred in the past.

Phylogenetic relationships among ITS1 sequences for all mite populations from this study plus sequences reported by Carew et al. (2009) are shown in Fig. 1. *A. tosichella* populations from KS, MT, SD, TX, and the Dundy1 population are clearly related to the Australian Type 1 of Carew et al. (2009). One additional Kansas population identified in GenBank (GU797252) is also included in this group (Fig. 1). Alternatively, the three Nebraska populations (NE, PREC, and Dundy2) are equivalent to the Australian Type 2 from Carew et al. (2009). *A. tosichella* appears to be a paraphylectic taxon in that *A. tulipae* clusters more closely with Type 1 mites than Type 2 mites. This suggests an ancestral hybridization event occurred between *A. tulipae* and *A. tosichella* Type 1 sometime after the divergence of *A. tosichella* Type 1 and Type 2 lineages. The ITS sequences for the populations in this study were deposited in GenBank (JX087352-JX087362).

To gain further insights among *A. tulipae* and the two types of *A. tosichella*, mitochondrial DNA, partially spanning the COI and COII genes, was PCR amplified, cloned and sequenced. C1-J-2183 and C2-N-3661 allowed amplification, albeit with some difficulty likely because of sequence mismatches, of a 1.4 kb PCR product from the NE, TX, and MT *A. tosichella*, two populations of *A. tulipae* (ON, NE), and the *A. kendalli* population. From the sequences of these PCR products, two new primers COIB and COIIA (Table 1) were synthesized and used to also amplify a ≈0.65 kb product from the SD, KS, Dundy1, and Dundy2 *A. tosichella* populations.

Genetic distances among these COI/COII sequences are presented in Table 3. This COI/COII region was significantly more divergent among the *A. tulipae* and *A. tosichella* populations than was evident in ITS1 sequences. As with the ribosomal data (ITS1), the KS, MT, SD, TX, and Dundy1 *A. tosichella* populations were nearly identical; only the MT population showed any divergence (0.5%). This group diverged

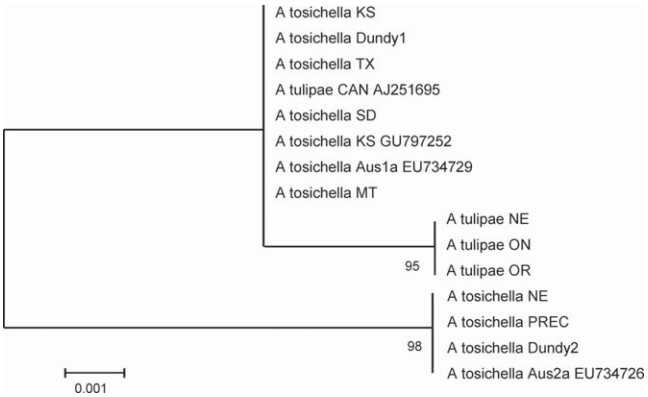

Fig. 1. Phylogenetic tree of eriophyid mite species and populations using the rDNA ITS1 region. Scale bar indicates percent genetic distances. Only nodes with bootstrap values 70% or greater (out of 1000 replicates) were retained.

≈13% from the NE and Dundy2 *A. tosichella* populations. The two *A. tulipae* populations were very similar (0.2% divergent) but diverged similarly from all *A. tosichella* populations (≈12–13%). Relatively consistent divergence was seen between *A. kendalli* and all the *A. tulipae* and *A. tosichella* populations tested (21–23%). The COI/COII sequences for the populations in this study were deposited in GenBank (JX102049-JX102058).

The COI/COII region provided a richer data set for differentiating the *A. tosichella* populations from each other and from *A. tulipae* (Fig. 2). A similar relationship was found with populations of twospotted spider mite where variation in the COI gene was ≈10-fold higher than that found in the ITS2 region (Navajas et al. 1998). In our analysis *A. tulipae* formed a monophyletic clade, as did all *A. tosichella* lineages, except the NE and Dundy 2 populations. These populations (NE, Dundy2) are clearly distinct from the others, while the KS, SD, TX, and Dundy1 populations are identical to each other. The Montana population comprises a sister clade to the latter three *A. tosichella* populations with high bootstrap support.

Samples of NE, TX, and MT populations of *A. tosichella*, the ON population of *A. tulipae*, plus the two Dundy County populations of *A. tosichella* were subjected to PCR using primers LR-J-12883, LR-N-12868, and COIR (Table 1). PCR amplification of mite samples with primers LR-N-12868 and COIR produced a 2.5 kb DNA fragment and established that the 16S gene was in the same orientation as the COI gene. This orientation is opposite that of most arachnids where 16S and COI are reversed (Black and Roehrdanz 1998, Fahrein et al. 2007). Following cloning and sequencing, BLAST searches of the GenBank database revealed that this 2.5 kb region of mtDNA contained the partial 16S gene, followed by genes encoding NADH dehydrogenase subunit two (NAD2), tRNA Met, tRNA Cys, and partial COI.

Finally, primers LR-J-12883 and SR-N-14588 (Table 1; Simon et al. 1994) were used in PCR of NE, MT, and TX, *A. tosichella* populations along with the ON *A. tulipae* population to produce a 1.3 kb product encoding partial 12S and 16S genes. The TX population for this segment differed by 0.2, 5.1, and 3.9% from the MT and NE populations and *A. tulipae* (ON), respectively, while the respective differences between the TX NAD2 and COI genes were 0.2, 16.9, and 13.5%, and 0.4, 14.0, and 11.7%. Interestingly, a partial COI gene sequence from mites identified as *A. tosichella* collected on *Bromus inermis* Leyss by Skoracka and Dabert (2010) was 12.1% divergent from *A. tulipae* (ON), 14.1% divergent from *A. tosichella* (TX; Type 1) and 12.6% divergent from *A. tosichella* (NE; Type 2). This suggests that additional lineages of *A. tosichella* will be found in the future. Skoracka and Dabert (2010) also reported two Polish *A. tulipae* populations that had

Table 3. Pairwise percent nucleotide differences among mite mitochondrial DNA COI/COII sequences for several *A. tosichella* and *A. tulipae* populations

	A. tosichella NE	*A. tosichella* Dundy2	*A. tosichella* KS	*A. tosichella* MT	*A. tosichella* SD	*A. tosichella* TX	*A. tosichella* Dundy1	*A. tulipae* ON	*A. tulipae* NE
A. tosichella Dundy2	0								
A. tosichella KS	13.1	13.1							
A. tosichella MT	13.2	13.2	0.5						
A. tosichella SD	13.1	13.1	0	0.5					
A. tosichella TX	13.1	13.1	0	0.5	0				
A. tosichella Dundy1	13.1	13.1	0	0.5	0	0			
A. tulipae ON	11.8	11.8	12.4	12.6	12.4	12.4	12.4		
A. tulipae NE	12.0	12.0	12.6	12.8	12.6	12.6	12.6	0.2	
A. kendalli	22.7	22.7	22.7	22.8	22.7	22.7	22.7	21.5	21.5

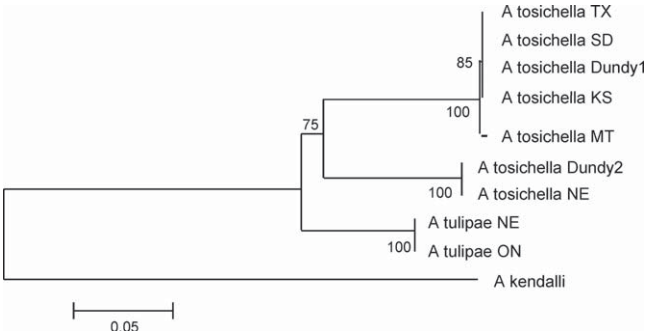

Fig. 2. Phylogenetic tree of *A. tulipae* and *A. tosichella* populations using the mtDNA COI/COII region. Scale bar indicates percent genetic distances. Only nodes with bootstrap values 70% or greater (out of 1000 replicates) were retained.

partial COI sequences that were 99.5% identical to the *A. tulipae* (ON) sequences reported here.

It is not surprising that the 16S rRNA gene is less divergent than the protein-coding genes, as rRNAs must maintain functional secondary structures. Nevertheless, amino acid sequences of proteins are likely constrained as well, and it is expected that there will be fewer nonsynonymous (amino acid replacement) differences per nonsynonymous site (dN) than synonymous differences per synonymous (silent) site (dS). In accordance with this expectation, we found that silent substitutions far outnumber replacement substitutions in the NAD2 and COI genes (Table 4). Analysis of partial COI-encoding sequences of *Abacarus hystrix* populations (Skoracka and Dabert 2010) revealed a similar excess of dS over dN substitution rates. Moreover, the high dS values reported in Table 4 suggests that the three mite lineages have been diverging for a considerable length of time.

Based on the mtDNA data, there appear to be multiple lineages of *A. tosichella* circulating in the Great Plains that clearly differ from *A. tulipae.* More populations from different geographical locations will need to be sampled to determine whether there is any biogeographical subdivision among lineages. The lack of diversity among the nuclear encoded ITS1 rDNA region raises the possibility that gene flow can occur between *A. tulipae* and *A. tosichella.* Additional nuclear gene markers and more extensive sampling will be required to address this question. Nevertheless, the mitochondrial sequence data suggests that if *A. tulipae* and *A. tosichella* are considered distinct species (Amrine and Stasny 1994), then it would be consistent to consider *A. tosichella*, Type 1 and Type 2 as possible separate species.

Table 4. Percent nonsynonymous differences per nonsynonymous site (dN) and percent synonymous differences per synonymous site (dS) for paired mite comparisons

Comparison	Gene			
	NAD2		COI	
	dN	dS	dN	dS
A. tosichella TX vs *A. tosichella* NE	7.1	53.3	16.9	71.4
A. tosichella TX vs *A. tulipae*	5.7	43.0	13.8	48.9
A. tosichella NE vs *A. tulipae*	7.0	45.3	15.8	40.9

The studies described here demonstrate that the genetic variability of *A. tosichella* populations parallels the biological diversity that has been demonstrated for these populations. These genetic differences correspond to differences in the mites' response to different resistant genes found in wheat (i.e., biotypes) shown by Harvey et al. (1999). The genetic differences also correlate with the ability of these mite populations to transmit HPV as described by Seifers et al. (2002). Seifers et al. (2002) found the NE population (Type 2) to be the most effective vector of HPV and the remaining populations (Type 1) to be much poorer vectors. However, the MT population did produce intermediate rates of HPV transmission when combined with WSMV. The MT population also showed a unique response pattern to mite-resistant wheat genes (Harvey et al. 1999), indicating unique biotypic characteristics. Our genetic data show a slight but consistent separation of the MT population from the remaining populations of Type 1 mites. The biological significance of these genetic differences needs to better understood. In addition to the HPV transmission differences, Seifers et al. (2002) showed that all the populations they tested provided adequate transmission of WSMV. However, Schiffer et al. (2009) indicate that only the Type 1 mite population they tested was able to transmit WSMV. These examples underscore the need for a better understanding of the biological differences both between and within these mite groups.

It is important that continued research be targeted to more clearly establish the biological and genetic characteristics of these wheat curl mite types and determine the extent of additional variability that may be present across geographic locations. The results of this study demonstrate the importance of accurately characterizing the type of wheat curl mites that are present and being used in detailed studies. The relationships between specific mite types and interactions with their vectored viruses hold important implications for understanding the epidemiology of this virus complex in the field and its future management.

Acknowledgments

This project was partially funded by U.S. Department of Agriculture (USDA) NC-integrated pest management (IPM) (CSREES) Grant 2004-34103-14443 and by USDA-Agriculture Research Services Specific Cooperative Agreement 58-5440-7-334. We thank D. Seifers and the late T. Harvey (Kansas State Univ.) for supplying their original colonies of *A. tosichella* for study and C. Bubl (Oregon State Univ. Extension) and J. Braun (Leamington, ON) for the original supply of *A. tulipae* mites. We also thank John Thomas for his technical assistance.

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Received 19 December 2011; accepted 10 July 2012.