Assessment of Variation Among *Thrips tabaci* Populations from Georgia and Peru Based on Polymorphisms in Mitochondrial Cytochrome Oxidase I and Ribosomal ITS2 Sequences[1]

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J. Entomol. Sci. 46(3): 191-203 (July 2011)

Abstract The onion thrips, *Thrips tabaci* (Lindeman), is the only known vector of *Iris yellow spot virus* (IYSV). IYSV was detected in Georgia for the first time in 2003. Phylogenetic analysis using nucleotide sequences of the IYSV capsid gene indicated that it may have been accidentally introduced from repackaging of imported Peruvian onions in the Vidalia onion-growing region. The tobacco thrips, *Frankliniella fusca* (Hinds), has been the dominant thrips species on onions in Georgia. However, in recent years the incidence of *T. tabaci* on onions has been consistently increasing. Laboratory competition studies indicated that *T. tabaci* outcompeted *F. fusca* on onion foliage. This led to speculation that a new biotype of *T. tabaci* may have been introduced along with IYSV through importation of Peruvian onions. This hypothesis was tested by analyzing variations in the mitochondrial cytochrome oxidase I gene and internal transcribed spacer region 2 of *T. tabaci* populations from Georgia and Peru. DNA was extracted from *T. tabaci* samples from Georgia and Peru and subjected to PCR using specific primers. The resulting amplicons were sequenced. Parsimony and Bayesian analysis of the COI sequences indicated that all the Peruvian taxa fell into a single clade along with one Georgia taxon. All the other Georgia taxa were in a separate clade. ITS2 sequence comparisons indicated that Georgia and Peru taxa were found in numerous clades. High variation among taxa from each region indicated that ITS2 may not be suitable to assess intraspecific variation among *T. tabaci* populations.

Key Words onion thrips, intraspecific variation, molecular markers

Onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), is the only known vector of *Iris yellow spot virus* (Family *Bunyaviridae*, Genus *Tospovirus*) (Kritzman et al. 2001). In comparison with other well-known tospoviruses, *Iris yellow spot virus* (IYSV) was only relatively recently described and information available on the vector(s) and IYSV transmission is very limited (Cortes et al. 1998). IYSV can severely affect bulb and seed onion, *Allium cepa* (L.), production as well as production of other members of the Alliaceae, such as garlic (*A. sativum* [L.]), leek (*A. porrum* [L.]), and chive (*A. schoenoprasum* [L.]) (Gent et al. 2006). IYSV was detected for the first time in 2003 in Georgia at an onion cull pile in the Vidalia onion-growing region (Mullis et al. 2004).

[1]Received 08 September 2010; accepted for publication 10 December 2010.
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Subsequent phylogenetic studies using the partial N-gene sequences indicated that the IYSV isolates in Georgia were similar (up to 99% identical at the nucleotide level) to IYSV isolates from Peru. However, there was a higher percentage of divergence between Georgia and Peru IYSV isolates from other regions in the United States, Asia, and Europe (Nischwitz et al. 2007). This indicated that IYSV was likely introduced into the Vidalia area through imported onions from Peru. Onions are regularly imported from Peru during the off-season by the packaging industry in the Vidalia area and re-packaged. During this process the rejected onions are dumped into nearby cull piles. IYSV was also detected from one such cull pile in the Vidalia area iterating that IYSV was likely introduced from Peru to Georgia (Mullis et al. 2004, Nischwitz et al. 2007).

The tobacco thrips, *Frankliniella fusca* (Hinds) (Thysanoptera: Thripidae), has been the dominant thrips species on onions in Georgia with *T. tabaci* representing <1% of pest thrips (Riley and Batal 1998). However, since 2004 *T. tabaci* population densities on onions have been consistently increasing (Sparks et al. 2011). Laboratory competition studies indicated that *T. tabaci* collected from Peruvian cull piles outcompeted native *F. fusca* on onions (Chitturi 2010). These observations led to speculation that a highly competitive *T. tabaci* biotype/strain may have been accidentally introduced into Georgia through imported Peruvian onions. The purpose of this study was to find evidence or lack thereof for such an introduction by examining intraspecific variation between *T. tabaci* populations from Georgia and Peru.

Molecular markers have been widely used to assess interspecific variation in Thysanoptera, examples include internal transcribed spacers (ITS1 and 2) (Moritz et al. 2001, Toda and Komazaki 2002), mitochondrial cytochrome oxidase (COI) (Brunner et al. 2002, Frey and Frey 2004), random amplified polymorphic DNAs (Bayar et al. 2002), and rDNA markers (Inoue and Sakurai 2007, Hoddle et al. 2008). To assess variation among organisms in lower taxonomic levels, it is often necessary to use molecular markers that target rapidly evolving gene regions (Hwang and Kim 1999). Mitochondrial DNA is known to evolve at a rapid rate when compared with the nuclear DNA due to which mitochondrial protein coding genes (COI and COII) are routinely used to assess genetic variation in lower taxonomic levels such as families, genera, species or populations (Frey and Frey 2004, Morris and Mound 2004, Asokan et al. 2007). Spacer regions such as ITS1 and 2 of rDNA due to their high variability also have been useful in assessing genetic variation in lower taxonomic levels (Moritz et al. 2001, Toda and Komazaki 2002, Rugman-Jones et al. 2006).

In an attempt to explain the recently observed spike in *T. tabaci* populations in Georgia onions, we used COI and ITS2 as molecular markers to evaluate genetic similarities between *T. tabaci* populations from Georgia and Peru. Taxa from other regions in the United States and elsewhere also were included in the process.

Materials and Methods

***Thrips tabaci* sample collection.** *Thrips tabaci* samples were collected from various locations in Georgia and in Peru from February to June 2008. Samples were collected from Tifton, Alpharetta, and from Tattnall Co. in Georgia. In Peru, they were collected from onion-growing regions along the western coast north and south of Lima. *Thrips tabaci* samples from New York and Kentucky were collected by Dr. Rick Bessin and Dr. Brian Nault. All thrips used for this study, except for samples from Alpharetta, were collected from onions. *Thrips tabaci* from Alpharetta were collected from basil, *Ocimum basilicum* (L.) plants. Voucher specimens from all collection sites are currently

maintained at the Thrips as Vectors of Agricultural Commodities (TVAC) Laboratory, University of Georgia, Tifton. Thrips were collected in 70% ethanol and stored at -20°C until further processing.

DNA extraction and PCR amplification. Only female thrips were used in this study. Total DNA was extracted from individual thrips using a modified salting-out protocol (Miller et al. 1988). Frozen thrips in 1.5-ml microfuge tubes were ground in 100 μL of extraction buffer (0.4M NaCl, 10mM Tris (pH 8), 2mM EDTA (pH 8)), to which 20 μL of 20% SDS and 2.5 μL of proteinase K (20mg/ml) were added and mixed by inverting. The tubes were incubated at 56°C for 3h, and 75 μL of 6M NaCl was added and centrifuged at 14,000 rpm for 30 min. The supernatant was transferred to a new tube and 100 μL of ice-cold isopropanol was added and stored at -20°C for 1 h followed by centrifugation at 12,000 rpm for 5 min at 4°C. The pellet was washed twice with ice-cold 70% ethanol and resuspended in 15 μL ultrapure double distilled H_2O free of nucleases and stored at -20°C indefinitely. The total DNA was directly used for PCR.

The reaction mixture for PCR had a total volume of 25 μL and contained 12.5 μL of the GoTaq buffer (Promega Inc, Madison, WI), 0.5 μL dNTP mix (Promega), 0.5 μL of forward and reverse primers (10 μM/μl), 0.2 of μl Taq polymerase (5units/μl), 2 μL of the DNA template, and nuclease free distilled water to obtain the total volume. The COI sequence was amplified using the following primers MtD-7.2F (5′-ATT AGG AGC HCC YAT AGC ATT-3′) and MtD-9.2R (5′-CAG GCA AGA TTA AAA TAT AAA CTT CTG-3′) (Brunner et al. 2002). Amplifications were conducted in an automated thermocycler (Perkin Elmer®, GeneAmp 2400, Waltham, MS) with the following conditions: 1 cycle of denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 48°C for 40 sec, and extension at 72°C for 1 min, and 1 cycle of final extension at 72°C for 10 min. The ITS2 region (flanked by partial segments of 5.8S rRNA and 28S rRNA genes) was amplified using the following primers 28Z (5′-AGA CTC CTT GGT CCG TGT TTC-3′) (Hillis and Dixon 1991) and P1 (5′-ATC ACT CGG CTC GTG GAT CG-3′) (Severini et al. 1996). Amplifications were conducted as previously described with the following conditions: 1 cycle of denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 57°C for 45 sec, extension at 72°C for 2 min, and one cycle of final extension at 72°C for 4 min.

Electrophoresis and sequencing. Electrophoresis of the amplicons was conducted on a 1% agarose gel in 0.5x TAE buffer and visualized with 0.01% ethidium bromide under UV light. Approximately 470 and 1300 bp bands corresponding to COI and ITS2, respectively, were excised under UV light and purified using a PCR purification kit (Qiagen, Inc., Valencia, CA) following the manufacturer's instructions. The purified products were sequenced in both directions at the University of California, Riverside, Genomics Core Instrumentation Facility using an Applied Biosystems 3,730 DNA analyzer with a Big-Dye version 3.1 kit (Applied Biosystems®, Foster City, CA).

Sequence alignment and phylogenetic analyses. Totals of 28 COI and 21 ITS2 sequences were used for phylogenetic analyses (Table 1). The sequences were aligned using CLUSTAL X and corrected by sight after setting the parameters for pairwise alignment (gap opening =10, gap extension =0.10), and multiple alignment (gap opening =10, gap extension = 0.20, delayed divergence = 30%) (Hall 2001, Jeyaprakash and Hoy 2009). The aligned sequences were used for phylogenetic analyses. Outgroup taxa (*Frankliniella tenuicornis* (Uzel), *Limothrips denticornis* (Haliday), *Aeolothrips* sp. and *Kladothrips* sp.) sequences and other related taxa sequences were obtained from GenBank. All the sequences used in this study are listed in Table 1.

Table 1. List of nucleotide sequences of taxa used in the study

Sequence type	Sequence name	Collection locale	Date of collection	GenBank accession number
mt COI	P_1	Peru	Feb 2008	JF429852
	P_2	Peru	Feb 2008	JF429853
	P_3	Peru	Feb 2008	JF429854
	P_4	Peru	Mar 2008	JF429855
	P_5	Peru	Mar 2008	JF429856
	P_6	Peru	Mar 2008	JF429857
	P_7	Peru	Mar 2008	JF429858
	P_8	Peru	Feb 2008	JF429859
	P_9	Peru	Feb 2008	JF429860
	Ti_1	Tifton, GA	Feb 2008	JF429861
	Ti_2	Tifton, GA	Feb 2008	JF429862
	Ti_3	Tifton, GA	Feb 2008	JF429863
	Ti_4	Tifton, GA	Mar 2008	JF429864
	A_1	Alpharetta, GA	Mar 2008	JF429865
	A_2	Alpharetta, GA	Mar 2008	JF429866
	A_3	Alpharetta, GA	Mar 2008	JF429867
	Ta_1	Tattnall Co, GA	Mar 2008	JF429868
	Ta_2	Tattnall Co, GA	Mar 2008	JF429869
	Ta_3	Tattnall Co, GA	Mar 2008	JF429870
	KY_1	Kentucky	June 2008	JF429871
	KY_2	Kentucky	June 2008	JF429872
	NY_1	New York	June 2008	JF429873
	NY_2	New York	June 2008	JF429874
	E_1	United Kingdom	GenBank	AM932020
	E_2	United Kingdom	GenBank	AM932043
	NZ	New Zealand	GenBank	EF591480
	Aeolothrips sp. (Ae)	Canada	GenBank	AAU42192
	Kladothrips sp. (K1)	Canada	GenBank	U93513
ITS 2	P_1	Peru	Feb 2008	JF429875
	P_2	Peru	Feb 2008	JF429876

Table 1. Continued

Sequence type	Sequence name	Collection locale	Date of collection	GenBank accession number
	P_3	Peru	Feb 2008	JF429877
	P_4	Peru	Feb 2008	JF429878
	P_5	Peru	Mar 2008	JF429879
	P_6	Peru	Mar 2008	JF429880
	P_7	Peru	Mar 2008	JF429881
	P_8	Peru	Mar 2008	JF429882
	P_9	Peru	Mar 2008	JF429883
	A_1	Alpharetta, GA	Mar 2008	JF429884
	A_2	Alpharetta, GA	Mar 2008	JF429885
	A_3	Alpharetta, GA	Mar 2008	JF429886
	Ta_1	Tattnall, County, GA	Mar 2008	JF429887
	Ta_2	Tattnall, County, GA	Mar 2008	JF429888
	Ta_3	Tattnall, County, GA	Mar 2008	JF429889
	KY_1	Kentucky	June 2008	JF429890
	KY_2	Kentucky	June 2008	JF429891
	NY_1	New York	June 2008	JF429892
	NY_2	New York	June 2008	JF429893
	Frankliniella tenuicornis (FT)	Germany	GenBank	AJ308592
	Limothrips denticornis (LD)	Germany	GenBank	AJ308594

Phylogenetic analyses were performed by using a MacBook (OS X) with a 2 GHz Intel Core 2 Dual processor. Parsimony and Bayesian analysis were performed for each type of data set. Parsimony analysis was performed in PAUP 4.0 (Swofford 2003). The COI data matrix included 28 taxa and 509 characters. All characters were considered unordered and of equal weight, 256 characters were considered constant, 99 characters were parsimony uninformative and 154 were parsimony informative, and the gaps were treated as 'missing'. Bootstrap analysis (2000 replicates) was done by stepwise addition and by using the branch swapping algorithm: tree-bisection-reconnection. Similarly, the ITS2 data matrix was also subjected to parsimony analysis. This matrix included 21 taxa and 1700 characters; 1078 characters were held constant, 288 variable characters were parsimony uninformative and 334 were parsimony informative.

Bayesian analysis was conducted by using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). To select the best fit model for each data set jModeltest 0.1.1

(Posada 2008) was used. The likelihood scores were computed along with Akaike Information Criterion (AIC) parameters. The parameters obtained for the best-suited model were used to preset the analysis in MrBayes. Bayesian Markov Chain Monte Carlo (MCMC) analysis was performed using the set priors Ngen=100000, nchains=4, sump burnin=2500, sumt burnin=2500. Twenty-five percent of the trees were eliminated and posterior probabilities were obtained. The likelihood parameters used in Bayesian analysis for each data set are listed in Table 2.

Results

Phylogenetic analysis of mitochondrial cytochrome oxidase I. Parsimony and Bayesian analyses revealed differences among *T. tabaci* populations. Bootstrap 50% majority-rule consensus tree for COI sequences representing the single most parsimonius tree is illustrated in Fig. 1a. Also represented is the clade credibility tree with posterior probability values obtained from Bayesian analysis (Fig. 1b). Two COI sequences belonging to 2 outgroup taxa *Kladothrips* sp. (Tubulifera: Phlaeothripidae) and *Aeolothrips* sp. (Terebrantia: Aeolothripidae) branched out from the remainder of the taxa. Variations in methods of phylogenetic analysis only contributed to minor differences.

Table 2. Model parameters for COI and the ITS data sets

Parameters	COI	ITS2
Model selected	TPM1uf+I+G	GTR+I+G
Partition	012210	012345
-lnL	2651.6245	6798.1422
K	61	50
freqA	0.3183	0.2298
freqC	0.1635	0.2751
freqG	0.1392	0.2539
freqT	0.3790	0.2413
R(a){AC}	1.0000	0.7778
R(b) AG}	5.5361	2.7567
R(c){AT}	1.9111	0.4638
R(d){CG}	1.9111	0.4757
R(e){CT}	5.5361	1.5089
R(f){GT}	1.0000	1.0000
P-inv	0.1890	0.2370
Gamma Shape	0.5090	0.1460

*Parameters for likelihood analysis were obtained from jModeltest output. Model selected was the best fit model from 88 candidate models. "lnL" is the loglikelihood of observing the alignment under the chosen model. "F" refers to the base frequency. "R" refers to the rates between the listed nucleotides. "P-inv" refers to proportion of sites assumed to be invariable.

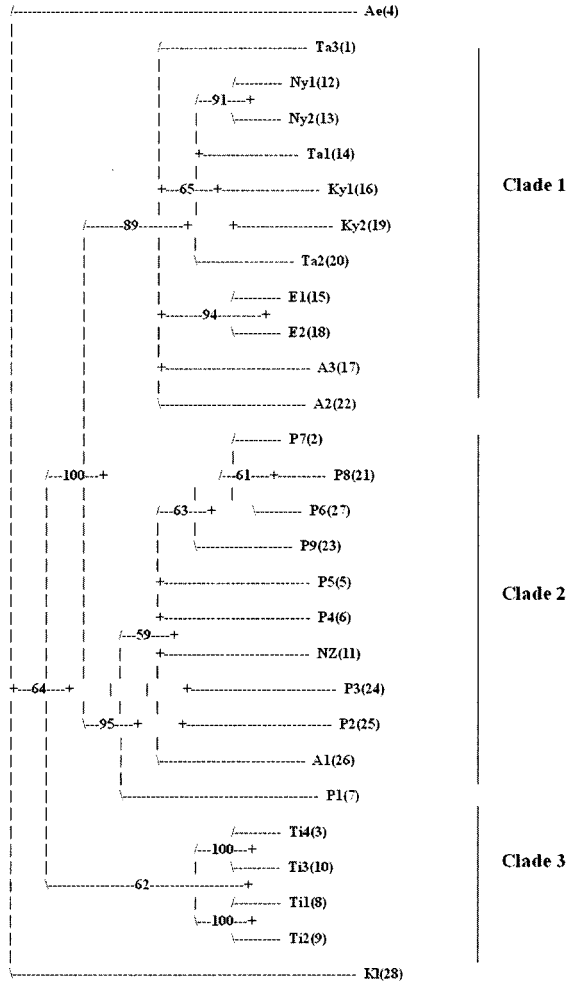

Fig. 1 a. Parsimony tree based on nucleotide sequences of the COI gene of Thrips tabaci obtained from Parsimony analysis. Bootstrap values greater than 50% are shown based on 2000 replications.

All 9 Peruvian taxa were found in a single clade along with 1 taxon from New Zealand and 1 taxon from Alpharetta, GA (A_1). This clade also was supported by high bootstrap and clade credibility values (≥95%). The remaining taxa from GA were found in a separate clade with taxa from New York, KY and the United Kingdom. Surprisingly, all *T. tabaci* collected from Tifton were found in a separate clade. The separation of all the taxa from Peru in a single clade indicates that genetic differences exist among *T. tabaci* populations from various locations. One taxon from Alpharetta was in a clade with 9 taxa from Peru. None of the Peruvian taxa were seen in other clades.

Phylogenetic analysis of ITS2 region. Bootstrap 50% majority-rule consensus tree for ITS2 sequences representing the single most parsimonius tree is illustrated in Fig. 2a. Also, represented is the clade credibility tree with posterior probability values

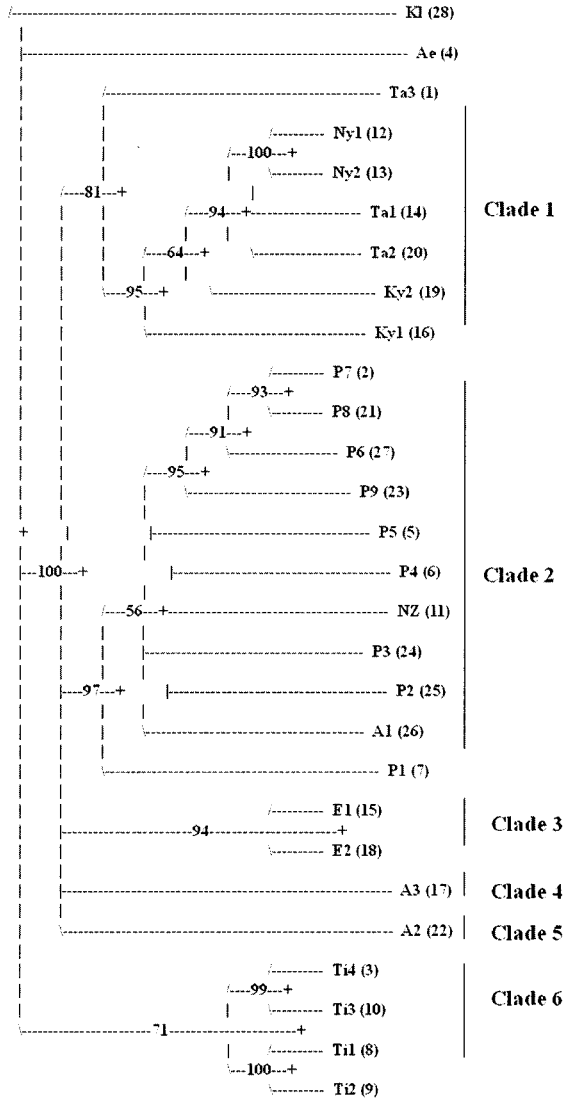

Fig. 1 b. Clade credibility tree based on nucleotide sequences of the COI gene of Thrips tabaci obtained from Bayesian Markovian Chain Monte Carlo (MCMC) analysis. Posterior probability values greater than 50% are shown based on 1 million generations.

obtained from Bayesian analysis (Fig. 2b). ITS2 sequences of *Frankliniella tenuicornis* (Uzel), and *Limothrips denticornis* (Haliday) (Thysanoptera: Thripidae) were treated as outgroups. Parsimony analysis revealed that *T. tabaci* sequences separated out into multiple clades from a single node. Single taxon was recorded in several clades; they included taxa from Alpharetta, Tattnall Co., Peru, NY, and Kentucky. Parsimony analysis

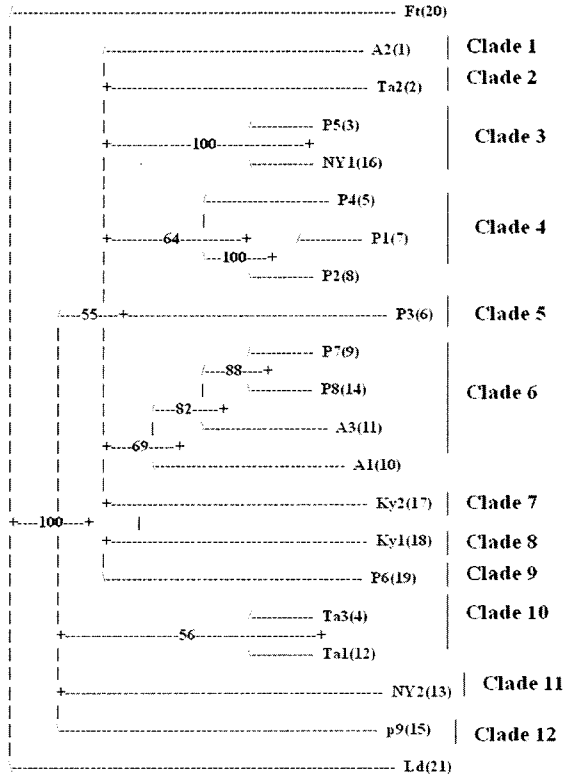

Fig. 2 a. Parsimony tree based on nucleotide sequences of the ITS2 region of *Thrips tabaci* obtained from Parsimony analysis. Bootstrap values greater than 50% are shown based on 2000 replications.

also revealed that taxa from Peru were found in multiple clades, including a clade with only taxa from Peru (clade 4). Peruvian taxa were found with taxa from New York (clade 3) and Alpharetta, GA (clade 6). Bayesian analysis, however, yielded slightly different results. As in the case of parsimony analysis only a single clade contained taxa exclusively from Peru (clade 5). The other 4 clades represented taxa from Peru along with taxa from Georgia, NY, and Kentucky. Unlike the COI phylogenetic trees which included all the taxa from Peru in a single clade, ITS2 trees included Peruvian taxa in multiple clades along with taxa from various other regions.

Discussion

Numerous morphological characters including antennal segments, wing setae, ocellar setae, notal setae, abdominal tergites, and sternites have been used widely in thrips taxonomy (Mound and Morris 2007). This approach requires extensive training and expertise in the area; yet, they may not be useful in differentiating taxa that belong to lower taxonomic classes, such as subspecies and populations. In these cases molecular markers are extremely useful. Molecular markers targeting rapidly evolving

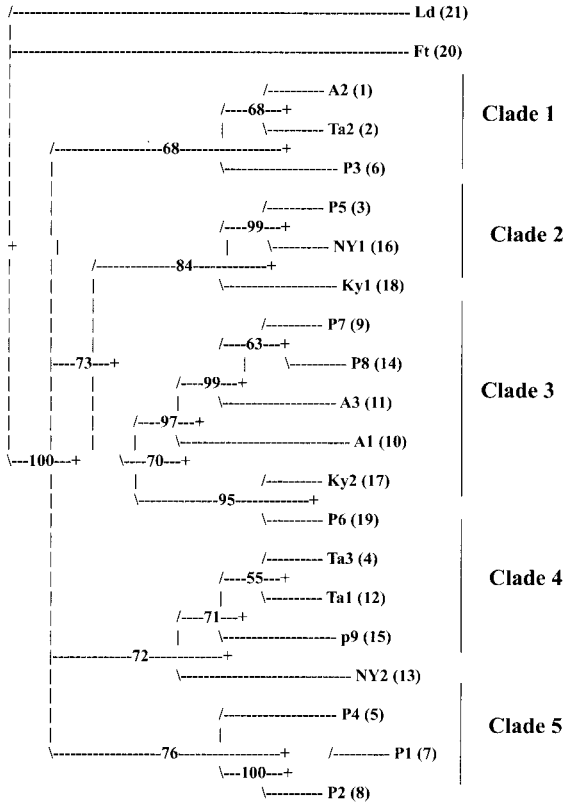

Fig. 2 b. Clade credibility tree based on nucleotide sequences of the ITS2 region of *Thrips tabaci* obtained from Bayesian Markovian Chain Monte Carlo (MCMC) analysis. Posterior probability values greater than 50% are shown based on 1 million generations.

regions have been particularly useful in identifying differences at species level. Moritz et al. (2001) used ITS region sequences to explain interspecific variability among various thrips species. Brunner et al. (2002) and Asokan et al. (2007) used variation occurring in DNA to differentiate several economically important thrips species. Hoddle et al. (2008) also used variations in the COI and 28S-D2 regions to distinguish 18 species of *Scirtothrips*. However, the applicability of these markers to assess intraspecific variation among populations remains relatively uncertain. Morris and Mound (2004) tested 2 populations of *Scirtothrips aurantii* (Faure) from Australia and South Africa on different host plants (Bromeliad and Citrus) using molecular markers targeting the mitochondrial and ITS DNA. Phylogenetic analysis revealed no clear separation between populations congruent to host plant or country of origin and concluded that the Australian population was not different from the South African population.

Thrips tabaci mitochondrial COI sequence analysis indicated that all the taxa from Peru were found in a single clade, and only 1 taxon from Alpharetta was found in the

Peruvian clade. The other taxa from Georgia were found along with taxa from Kentucky and New York. The separation of all the Peruvian taxa in a single clade and the Georgia taxa in multiple clades indicates that there may have been multiple introductions of *T. tabaci* from several places into Georgia. The presence of taxa from Georgia, NY, KY, and the United Kingdom in the same clade iterates that there may have been multiple introductions into Georgia including from Peru. The presence of a single taxon from Georgia in the Peruvian clade suggests that it may have been introduced from Peru. However, the taxa from Tattnall Co., which is believed to be the point of IYSV and *T. tabaci* introduction to Georgia, were present in a different clade. These results suggest that the *T. tabaci* may have been introduced into Georgia through the imported onions, but did not establish itself in Georgia or that native thrips populations outcompeted the Peruvian biotype. Nevertheless, it does not offer a plausible explanation for the recent spike in *T. tabaci* incidence in Georgia.

ITS2 sequence analyses indicated that members from Georgia and Peru were in the same clade; however, the taxa from Peru were observed in multiple clades suggesting that there is high variability within the populations from Peru. Morris and Mound (2004) also were not able to ascertain differences between populations of *S. aurantii* using ITS DNA. They attributed it to the presence of multiple copies of the spacer in some populations. The multiple copies of ITS2 could have also caused the high variability within *T. tabaci* populations and needs to be investigated further.

Phylogenetic analysis of the IYSV N-gene revealed that the isolates from Georgia and Peru were in the same clade. The partial N-gene sequences from both Peru and Georgia were up to 99% identical at the nucleotide level (Nischwitz et al. 2007). On the contrary, phylogenetic analysis of the *T. tabaci* COI and ITS2 sequences did not reveal a clear distinction between the 2 populations. It is not illogical to assume that the virus and its vector could have been introduced from the same onion shipment. Thrips are known to feed on the necks of onion bulbs and could also have been introduced at the same time as that of IYSV. *Thrips tabaci* COI sequence analysis indicates that such an introduction could have occurred, but only one taxon from Alpharetta, GA, was in the Peruvian COI clade. In the case of ITS2 analysis the Peruvian taxa by themselves exhibited extensive variability and were not found in a single clade. This could have been caused by the variation within individuals and due to multiple copies of ITS (Leo and Barker 2002, Morris and Mound 2004). The reliability of ITS2 to assess differences among populations of *T. tabaci* also needs to be reconsidered and the usefulness of other approaches needs to be examined. Though our results suggest that there may have been *T. tabaci* introductions from Peru into Georgia, they do not support the introduction through imported Peruvian onions. Clearly, more research is needed to address this important issue of thrips biotype introductions.

Acknowledgments

The authors thank Drs. Rick Bessin (Department of Entomology, University of Kentucky, Lexington, KY), Brian Nault (Department of Entomology, Cornell University, Geneva, NY), and others for providing thrips samples, and Ms. Kippy Lewis, and Mr. Stephen Mullis (Department of Plant Pathology, University of Georgia, Tifton, GA) for their assistance with DNA extraction and PCR. We also greatly appreciate Dr. Mike Toews (Department of Entomology, University of Georgia, Tifton, GA) and Dr. Tracie Jenkins (Department of Entomology, University of Georgia, Griffin, GA) for reviewing this manuscript and for their useful suggestions.

References Cited

Asokan, R., N. K. Krishna Kumar, V. Kumar and H. R. Ranganath. 2007. Molecular differences in the mitochondrial cytochrome oxidase I (mtCOI) gene and development of a species-specific marker for onion thrips, *Thrips tabaci* Lindeman, and melon thrips, *T. palmi* Karny (Thysanoptra: Thripidae), vectors of tospoviruses (Bunyaviridae). Bull. Entomol. Res. 97: 461-470.

Bayar, K., O. Torjek, E. Kiss, G. Gyulai and L. Heszky. 2002. Intra- and inter-specific molecular polymorphism of thrips species. Acta Biol. Hung. 53: 317-324.

Brunner, P. C., C. Flemming and J. E. Frey. 2002. Molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP based approach. Agric. For. Entomol. 4: 127-136.

Chitturi, A. 2010. *Thrips tabaci* and its interactions with I*ris yellow spot virus*. Ph.D. Thesis, Univ. Georgia, Athens.

Cortes, I., I. C. Liveratos, A. Derks, D. Peters and R. Kormelink. 1998. Molecular and serological characterization of *Iris yellow spot virus*, a new and distinct *Tospovirus* species. Phytopathology 88: 1276-1282.

Frey, J. E. and B. Frey. 2004. Origin of intra-individual variation in PCR-amplified mitochondrial cytochrome oxidase I of *Thrips tabaci* (Thysanoptera: Thripidae): mitochondrial heteroplasmy or nuclear integration. Hereditas 140: 92-98.

Gent, D. H., L. J. du Toit, S. F. Fichtner, S. K. Mohan, H. R. Pappu and H. F. Schwartz. 2006. *Iris yellow spot virus*: An emerging threat to onion bulb and seed production. Plant Dis. 90: 1468-1480.

Hall, B. G. 2001. Phylogenetic trees made easy. Sunderland, MA, Sinauer Associates.

Hillis, D. M. and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66: 411-453.

Hoddle, S. M., J. M. Heraty, P. F. Rugman-Jones, L. A. Mound and R. Southamer. 2008. Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. Ann. Entomol. Soc. Am. 101: 491-500.

Hwang, U. and W. Kim. 1999. General properties and phylogenetic utilities of nuclear ribosomal DNA and mitochondrial DNA commonly used in molecular systematics. Korean J. Parasitol. 37: 215-228.

Inoue, T. and T. Sakurai. 2007. The phylogeny of thrips (Thysanoptera: Thripidae) based on partial sequences of cytochrome oxidase I, 28S ribosomal DNA and elongation factor -1α and the association with vector competence of tospoviruses. Appl. Entomol. Zool. (Jpn.) 42: 71-81.

Jeyaprakash, A. and M. A. Hoy. 2009. First divergence time estimate of spiders, scorpions, mites, and ticks (subphylum: Chelicerata) inferred from mitochondrial phylogeny. Exp. Appl. Acarol. 47: 1-18.

Kritzman, A., M. Lampel, B. Raccah and A. Gera. 2001. Distribution and transmission of *Iris yellow spot virus*. Plant Dis. 85: 838-842.

Leo, N. P. and S. C. Barker. 2002. Intergenomic variation in ITS 2 rDNA in the louse of humans, *Pediculus humanus*: ITS2 is not a suitable marker for population studies in this species. Insect Mol. Biol. 11: 651-657.

Miller, S. A., D. D. Dykes and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16: 1215.

Moritz, G., M. Paulsen, C. Delker, S. Picl and S. Kumm. 2001. Identification of thrips using ITS-RFLP analysis, Pg. 365-367. *In* R. Marullo, and L.A. Mound, (eds.), Proc. the 7th International Symposium on Thysanoptera. Reggio, Calabria, Italy.

Morris, D. C. and L. A. Mound. 2004. Molecular relationships between populations of South African Citrus thrips (*Scirtothrips aurantii* Faure) in South Africa and Queensland, Australia. Aust. J. Entomol. 43: 353-358.

Mound, L. A. and D. C. Morris. 2007. The insect order Thysanoptera: classification versus systematics. Zootaxa 1668: 395-411.

Mullis, S. W., D. B. Langston, R. D. Gitaitis, J. L. Sherwood, A. C. Csinos, D. G. Riley, A. N. Sparks, R. L. Torrance and M. J. Cook IV. 2004. First report of Vidalia onion (*Allium cepa*) naturally infected with *Tomato spotted wilt virus* and *Iris yellow spot virus* (Family *Bunyaviridae*, genus *Tospovirus*). Plant Dis. 88: 1285.

Nischwitz, C., H. R. Pappu, S. W. Mullis, A. N. Sparks, D. R. Langston, A. S. Csinos and R. D. Gitaitis. 2007. Phylogenetic Analysis of *Iris yellow spot virus* isolates from Onion (*Allium cepa*) in Georgia (USA) and Peru. Phytopathol. Z. 155: 531-535.

Posada, D. 2008. jModelTest: Phylogenetic Model averaging. Mol. Biol. Evol. 25: 1253-1256.

Riley, D. G. and K. Batal. 1998. Management of thrips on onions, Pg.41-42. *In* Georgia Onion Research-Extension Report 1996-97. Univ. of Georgia, Coop. Research-Extension Publ. No. 3-98. http://www.caes.uga.edu/commodities/fruits/vidalia/publications.html.

Ronquist, F. and J. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Rugman-Jones, P. F., M. S. Hoddle, L. A. Mound and R. Southamer. 2006. Molecular identification key for pest species of *Scirtothrips* (Thysanoptera: Thripidae). J. Econ. Entomol. 99: 1813-1819.

Sparks, A. N., S. Diffie and D. G. Riley. 2011. Thrips species composition shift on onions in the Vidalia production region of Georgia. J. Entomol. Sci. 46: 40-46.

Severini, C., F. Silvestrini, P. Mancini, G. La Rosa and M. Marinucci. 1996. Sequence and secondary structure of the rDNA second internal transcribed spacer in the sibling species *Culex pipens* L. and *Cx. quinquefasciatus* Say (Diptera: Culicidae). Insect Mol. Biol. 5: 181-186.

Swofford, D. L. 2003. PAUP*. Phylogenetic Analysis using Parsimony (*and Other Methods), Version 4. Sunderland, MA, Sinauer Associates.

Toda, S. and S. Komazaki. 2002. Identification of thrips species (Thysanoptera: Thripidae) on Japanese fruit trees by polymerase chain reaction and restriction fragment length polymorphism of the ribosomal ITS2 region. Bull. Entomol. Res. 92: 359-363.