Thrips tabaci Population Genetic Structure and Polyploidy in Relation to Competency as a Vector of Tomato Spotted Wilt Virus

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Abstract

Knowledge of population-level genetic differences can help explain variation among populations of insect vectors in their role in the epidemiology of specific viruses. Variation in competency to transmit Tomato spotted wilt virus (TSWV) that exists among populations of Thrips tabaci has been associated with the presence of cryptic species that exhibit different modes of reproduction and host ranges. However, recent findings suggest that vector competency of T. tabaci at any given location depends on the thrips and virus populations that are present. This study characterizes the population genetic structure of T. tabaci collected from four locations in North Carolina and examines the relationship between population genetic structure and variation in TSWV transmission by T. tabaci. Mitochondrial COI sequence analysis revealed the presence of two genetically distinct groups with one characterized by thelytokous, parthenogenetic reproduction and the other by arrhenotokous, sexual reproduction. Using a set of 11 microsatellite markers that we developed to investigate T. tabaci population genetic structure, we identified 17 clonal groups and found significant genetic structuring among the four NC populations that corresponded to the geographic locations where the populations were collected. Application of microsatellite markers also led to the discovery of polyploidy in this species. All four populations contained tetraploid individuals, and three contained both diploid and tetraploid individuals. Analysis of variation in transmission of TSWV among isofemale lines initiated with individuals used in this study revealed that ‘clone assignment,’ ‘virus isolate’ and their interaction significantly influenced vector competency. These results highlight the importance of interactions between specific T. tabaci clonal types and specific TSWV isolates underlying transmission of TSWV by T. tabaci.

Introduction

Knowledge of population-level genetic differences and clonal diversity in sexual and asexual species has improved our understanding of the role insect vectors have in the epidemiology of specific viruses [1–6]. Thrips tabaci Lindeman, is a polyphagous insect pest species that is also a vector of two important plant infecting Tospoviruses (Genus: Tospovirus Family: Bunaviridae), Tomato spotted wilt virus (TSWV) and Iris yellow spot virus (IYSV) [7,8]. In nature tospoviruses are transmitted exclusively by only 14 of approximately 7,400 described thrips species [9,10]. Worldwide, T. tabaci is the sole vector of IYSV, which is responsible for an estimated U.S. $60–90 million in onion crop losses in the western U.S. [11], and is one of 10 reported thrips vectors of TSWV, estimated to cause over U.S. $1 billion in crop losses annually worldwide [12,13]. T. tabaci was the first species reported to transmit TSWV and was believed to be the primary vector until transmission studies revealed extensive variation in its competency as a vector of TSWV. Brazilian and Canadian populations of T. tabaci did not transmit TSWV in transmission assays [14–16], whereas in Europe both poor and efficiently transmitting populations are found, sometimes in the same area [17]. Despite this variation T. tabaci is the primary vector of TSWV in tobacco and vegetable production systems in Europe and Tasmania, respectively [17–21]. In the USA T. tabaci is not reported as an important vector of TSWV, however, both poor and efficiently transmitting populations have been observed [22,23].

Variation in transmission efficiency by T. tabaci populations has long been associated with the presence of cryptic species that exhibit different modes of reproduction and host ranges [17,24,25,26,27]. Zawirska [24] concluded there are two subspecies of T. tabaci, one exhibiting arthrotokous parthenogenesis that is found on tobacco and capable of transmitting TSWV, the other exhibiting thelytokous parthenogenesis that is not found on tobacco and not capable of transmitting TSWV. Two recent studies examining mitochondrial cytochrome oxidase subunit one (mtCOI) sequence variation have provided evidence for at least three genetically distinct groups based on host plant (leek versus tobacco) [26], or reproductive mode (male producing and thelytokous populations) [27]. It remains unknown whether or not ongoing gene flow occurs among these groups. Although inherent differences in vector competency of the different groups and their distributions may play an important role in TSWV
The objectives of this study were to characterize the population genetic structure of *T. tabaci* collected from four different locations in North Carolina using mtCOI sequences and microsatellite markers, and to examine whether there is a relationship between genetic structure and variation in TSWV transmission by *T. tabaci*. Isofemale lines established from parthenogenetically reproducing, field-collected females that were previously characterized for both reproductive mode type and their ability to transmit several TSWV isolates were used in this study [22]. In addition to characterizing genetic structure, clonal assignments based on allele similarities of microsatellite loci were incorporated into statistical models describing the relationships between TSWV isolate and thrips population combinations, and is more commonly observed when the tested TSWV isolate and thrips combinations were collected from the same location [22]. Additionally, there is evidence that efficient transmission of TSWV by *T. tabaci* is specific to certain isolate and thrips population combinations, and is more commonly observed when the tested TSWV isolate and thrips combinations were collected from the same location [22]. All of this suggests that defining *T. tabaci* as a TSWV vector on the basis of genetic groupings associated with host plant or reproductive mode alone is not sufficient for understanding the potential of this vector in disease epidemics.

The sex of thrips was verified visually under a compound light microscope and recorded for each individual before DNA extraction. Total genomic DNA was extracted from individual thrips using the DNeasy® Blood and Tissue Kit (QIAGEN, Valencia, CA) according to manufacturer’s instructions. DNA was eluted in 30 μl of AE buffer.

Mitochondrial COI sequence alignments were performed using the Vector NTI Advance 10 program (Invitrogen, Carlsbad, CA). Phylogenetic relationships were examined using Molecular Evolutionary Genetics Analysis (MEGA), Version 4 [33]. Neighbor-joining (NJ), UPGMA, and Minimum Evolution analyses in which all characters were equally weighted and 10,000 bootstrap replicates were conducted. Minimum spanning networks were also constructed to examine the evolutionary relationship between the mtDNA sequences using the program TCS 1.21 [34].

Microsatellite development and analysis

From five *T. tabaci* specimens, selected from geographically distant sampling locations, DNA was extracted using the DNEasy Blood & Tissue kit (Qiagen, Valencia, CA). DNA quality and concentration from each specimen were determined using the BioSpec-nano spectrophotometer (Shimadzu Scientific Instru-
Pooled DNA from the five specimens was then subjected to shotgun sequencing using the Roche 454 Genome Sequencer FLX (Roche Applied Science, Penzberg, Germany) with the Titanium Sequencing kit XLR 70, performed at the Genomic Sequencing Laboratory located at the North Carolina State University. Sequencing was performed on a 1/16 GS-FLX PTP.

A total of 97,611 reads were obtained with an average read length of 354 bp and a total of 34,514,909 bp. Using MSATCOMMANDER version 0.8.2 [35], all unassembled sequences were screened for di-, tri-, and tetranucleotides using default settings within the program. Primers were designed using the PRIMER3 software [36], implemented within the MSATCOMMANDER program, and tagged with a 19 base pair (bp) M13 forward label (CACGACGTTGTAAAACGAC). Amplification products were chosen to be within a 100 to 400 bp range (including M13 tag), with an optimal annealing temperature of 59°C (range 57°C–63°C), an optimal GC content of 50%, low levels of self- or pair-complementarity, and a maximum stability of 8.0 [35]. Following the removal of duplicate sequences, a total of 879 sequences were found to contain tandem repeats within the desired criteria with sufficient flanking region for primer design: 352 di-, 304 tri-, and 222 tetra-nucleotide microsatellites with at least 10, 5, and 5 repeats, respectively. Of these, 52 primer pairs were tested with 11 selected for microsatellite analysis (Table 2).

Reactions were subsequently denatured at 95°C for 4 min prior to loading onto a 25 cm 6% polyacrylamide gel, using either 50–350 bp or 50–700 bp IRDye standards (Li-Cor Inc., Lincoln, NE) for accurate product sizing. Results were analyzed using the GeneProfiler software (Scanalytics, Inc., BD Biosciences Bioimaging, Rockville, MD).

Clonality and polyploidy of NC Thrips tabaci populations had to be considered during microsatellite analyses. The T. tabaci individuals used in this study exhibited arrhenotoky and thelytoky, and females from NC were shown to be both diploid and polyploid during microsatellite marker development for this project [37]. Ploidy could not be determined for the specific individuals included in this study because storage of the thrips in 95% ethanol was not compatible with flow cytometry methods used for ploidy determination [37]. However, results from a flow cytometry and microsatellite marker analysis of other individuals taken from the isofemale lines maintained in the laboratory suggest that these populations are tetraploid based on the observed genome sizes of the polyploids, and the maximum number of microsatellite alleles per locus in individuals across North Carolina [37]. Additionally, it was shown that the maximum number of alleles observed with the microsatellite markers used in this study did provide reliable estimates of ploidy [37]. Therefore, ploidy estimates were generated based on maximum allele copy number. Female individuals exhibiting a maximum of two alleles across all loci were characterized as diploid and individuals with more than two bands at any locus were characterized as tetraploid [37]. Males included in this study had a maximum of one allele per locus.

The R program Polysat [38] was used for analysis because it can handle samples of mixed ploidy, provides methods to estimate allele copy number in partial heterozygote polyploids, and calculates clonal diversity statistics. Pairwise genetic Bruvo distances were then calculated using the method described by Bruvo et al. [39]. This pairwise genetic distance matrix was then used to perform a principle component analysis (PCA) to examine population structuring by plotting the first two principle components. Groups of asexually related individuals were assigned to clonal groups using the Bruvo distance matrix. A distance measure
of 0.2 was used for these assignments based on the histogram of the distribution of genetic Bruvo distances. Allele frequencies were then estimated using the simpleFreq function and pairwise $F_{ST}$ values calculated based on the methods of Nei [40]. Isolation by distance was also calculated based on pairwise $F_{ST}$ and distance matrices in Genepop using the isolede suboption with a Mantel's test with 1000 permutations, log transformations of distance and $F/(F-1)$-statistics [41].

| Isoline | Location | Host Plant | RM | Ploidy | Clone | mtCOI | GenBank Accession |
|---------|----------|------------|----|--------|-------|-------|------------------|
| Apex-1  | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 1 | 1 | JX403008 |
| Apex-2  | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |
| Apex-4* | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |
| Apex-6  | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |
| Apex-10 | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |
| Apex-11 | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |
| Apex-14 | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 3 | 4 | JX403011 |
| Apex-16 | Apex, NC: 35.718306N, –78.926981W | Allium cepa | T | 4 | 2 | — | — |

**Table 1. Thrips tabaci** samples collection information.

1Reproductive Mode.

2Based on microsatellite marker data.

3mtCOI haplotypes.

*characterized for transmission efficiency in [22].

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Population structure and TSWV transmission

A previous TSWV transmission study conducted with individuals from the same isofemale line showed that isolate, isofemale line and their interaction were statistically significant effects describing the probability of successful transmission of TSWV [22]. Additionally, higher transmission rates were seen among sympatric TSWV isolate-isofemale line pairings (collected from the same location) versus allopatric TSWV isolate-isofemale line pairings (collected from different locations). These results indicate that both viral and thrips genetic components underlie successful transmission of TSWV by *T. tabaci*, and suggest that local adaptation may be occurring between TSWV isolates and thrips.

To further investigate the virus-vector relationship between TSWV isolates and *T. tabaci*, the analysis of TSWV transmission was re-run using 12 isofemale lines that came from one of the four populations used in the genetic analysis for which both clonal assignments and TSWV transmission data in individuals were available, along with two additional isofemale lines, one from Kinston, NC and one from Cove City, NC, for which transmission and microsatellite clone assignment information was available. Not enough transmission data were available for the Mills River isofemale lines to include them in this analysis. Models were run using Proc Glimmix (SAS Institute, Cary, NC). The mtCOI haplotypes were not included in this analysis because the small number of arrhenotokous individuals available did not permit a robust comparison.

### Results

#### Genetic analysis

NJ, UPGMA, and Minimum Evolution phylogenetic methods employed in MEGA produced identical tree topologies (Figure 2). The individuals in these populations were subdivided into two groups that corresponded with their reproductive mode. Group one was comprised of all of the thelytokous individuals and group two was comprised of the arrhenotokous individuals. Within group sequence divergence estimates for groups one and two were 0.23% (±0.10%) and 0.16% (±0.08%), respectively. Overall mean sequence divergence estimates among samples were 1.74% (±0.36%), whereas between group mean sequence divergence was 3.42% (±0.72%), which supports the results from the phylogenetic analysis, and the previous analysis of Brunner et al. [26] that shows genetic variation sufficiently large to suggest these groups represent different subspecies or biotypes.

#### Population genetic analysis

All of the individuals collected from Apex, Jackson Springs, and Faison exhibited thelytokous parthenogenesis, and all of the...
individuals from Mills River exhibited arrhenotokous parthenogenesis. Both diploid and tetraploid females were found in Faison, Jackson Springs and Mills River collection sites, whereas only tetraploids were collected from Apex. Males from Mills River included in the study appeared to be haploid based on the maximum number of alleles observed at each microsatellite locus, however, ploidy has not been formally examined for male *T. tabaci* [37]. Mean allelic richness was four alleles/locus, and ranged from three-six when analyzed per locus and per population. A total of 17 clonal groups were identified from these four populations (Table 1). No arrhenotokous individual belonged to the same clonal group as a thelytokous individual, and both diploid and tetraploid individuals were found in the same clonal groups.

The principle component analysis conducted on the first two principle components of the genetic distance matrix calculated with the Bruvo distance measure (Figure 4), pairwise $F_{ST}$ (Table 3) and clonal assignments (Table 1) all suggest that populations are geographically structured across NC and that migration of individuals occurs among some of the locations. A total of 17 clonal groups were identified from these four populations (Table 1). No arrhenotokous individual belonged to the same clonal group as a thelytokous individual, and both diploid and tetraploid individuals were found in the same clonal groups.

Population structure and TSWV transmission

The probability of TSWV transmission based on the variables ‘virus isolate’, ‘isofemale line’ and ‘sympatry’ [22] was re-analyzed to further investigate the role of thrips genetic components in the transmission of TSWV by including genetic variables for clonal group calculated from microsatellite marker data. First, the statistical models used by Jacobson and Kennedy [22] were re-run on the subsample of isofemale lines that were included in the population genetic analysis. These analyses showed that the main effect of ‘isofemale line’ ($P = 0.0552$) was not significant, but ‘virus isolate’ ($P < 0.0001$) and ‘isofemale line x virus isolate’ interaction ($P = 0.0070$) were statistically significant with this subgroup. When the variable ‘sympatry’, replaced the interaction term in this model to investigate whether this interaction was significant among virus isolates and thrips isofemale lines that occur in the same location, ‘virus isolate’ ($P < 0.0001$) and ‘isofemale line’ ($P < 0.0001$) were significant, but ‘sympatry’ ($P = 0.4436$) was not significant.
Next, these models were run using genetic clonal groups, identified from microsatellite marker analysis, as the variable to describe the thrips isofemale lines. A comparison of transmission efficiency based on mtCOI haplotype could not be conducted due to the small sample sizes of transmission phenotypes available for arrhenotokous haplotypes. When ‘isofemale line’ was replaced with ‘clone assignment’ as the variable to model thrips transmission, ‘clone assignment’ (P < 0.0001) and ‘virus isolate’ (P < 0.0001) were significant main effects, but ‘sympatry’ was not (P = 0.9577). When ‘clone assignment’ (P < 0.0001), ‘virus isolate’ (P < 0.0001) and their interaction term (p = 0.0399) were included in the model, however, all three terms were statistically significant. These results highlight the importance of interactions between specific T. tabaci clonal types and specific TSWV isolates in determining transmission of TSWV by T. tabaci.

Discussion

The first objective of this study was to characterize the population genetic structure of T. tabaci collected from four different locations in NC using mtCOI sequences and microsatellite markers. The results of the mtCOI sequence analysis support previous evidence for the existence of genetically distinct groups within this species [26,27], and show that two genetically distinct groups are present in NC (Figure 2). The minimum spanning haplotype networks also show strong differentiation between reproductive types (Figure 3) and support the results of the phylogenetic analysis.

This is the first study to examine the population genetic structuring of T. tabaci populations using microsatellite markers. Microsatellite markers developed here revealed genetic structure among the four NC populations that corresponded to both the geographic locations where the populations were collected, and the grouping of individuals observed in the phylogenetic analysis conducted with mtCOI sequences. Pairwise FST values ranged from 0.06–0.20 indicating moderate genetic differentiation between all populations (Table 3). The PCA and clonal assignments suggest that arrhenotokous and thelytokous populations are reproductively isolated from each other because arrhenotokous and thelytokous populations do not cluster together or share clonal types. Isolation by distance was not significant among these populations (Figure 5), however this could be due to a small population sample size. Results suggesting reproductive isolation may also be confounded by distance and geographic barriers. North Carolina is comprised of three geographic regions that vary in climate and topography; the mountains, the piedmont and the coastal region. Clonal admixture only occurred among the three thelytokous populations that were collected in the central piedmont and coastal region. These three populations were separated by 142 km or less, suggesting that dispersal occurs at this distance. The arrhenotokous population was collected in a field located in the Appalachian Mountains, and was therefore separated from the thelytokous populations by two potential geographic barriers to dispersal and gene flow: the mountains, and the 261–402 km distance that separate them. Additional studies of population structure should be conducted where thelytokous and arrhenotokous individuals coexist to further investigate breeding structure of local populations and the potential for interbreeding between populations with different reproductive modes.
The discovery of polyploidy in *T. tabaci* populations during microsatellite marker development was unexpected, and is the first report of polyploidy in Thysanoptera [37]. NC populations of *T. tabaci* appeared to be diploid and tetraploid based on genome size estimates for multiple individuals. Because we did not expect populations to be polyploid our sample collection, storage, and processing methodology only allowed for the determination of ploidy and allele dosage in these populations using allele frequency based estimation methods. Based on these methods, all populations sampled contained tetraploid individuals, and three of the populations were comprised of both diploid and tetraploid individuals. Polyploidy represents a significant barrier to the establishment or transition to asexual/parthenogenetic reproduction [44]. Following the establishment of a facultatively parthenogenetic phase (asexual reproduction by a sexually reproducing species), populations may then be established through inbreeding. Outbreeding with diploids, in contrast, yielding triploid offspring, is considered a reproductive dead-end due to the increased likelihood of reduced fertility [44]. Polyploidy, and thus clonality have been thought to contribute to the successful spread and establishment of individuals into marginal habitats. Research examining the effects of both clonality and polyploidy among arthropods showed that in some species the abundance and distribution of clones is better described when geographic patterns of polyploidy are examined alone, or with distribution of parthenogens [45]. Thelytokous *T. tabaci* are more common worldwide; males and arrhenotokous populations are

![Figure 5. Scatter plot of transformed pairwise $F_{ST}$ values and pairwise geographic distances between North Carolina populations of *T. tabaci* with regression line, $R^2$ value and $P$ value from a Mantel’s test for isolation by distance.](https://doi.org/10.1371/journal.pone.0054484.g005)

**Table 3.** Pairwise $F_{ST}$ and pairwise distances (km) between four NC populations of *T. tabaci.*

| Pairwise $F_{ST}$ | Apex | Faison | Mills River | Jackson Springs |
|-------------------|------|--------|-------------|-----------------|
| Apex              | 0.00 |        |             |                 |
| Faison            | 0.17 | 0.00   |             |                 |
| Mills River       | 0.14 | 0.20   | 0.00        |                 |
| Jackson Springs   | 0.09 | 0.06   | 0.13        | 0.00            |

| Pairwise Distance (km) | Apex | Faison | Mills River | Jackson Springs |
|------------------------|------|--------|-------------|-----------------|
| Apex                   | 0.00 |        |             |                 |
| Faison                 | 96.00| 0.00   |             |                 |
| Mills River            | 332.00| 402.00| 0.00        |                 |
| Jackson Springs        | 90.00| 142.00| 261.00      | 0.00            |

*Reproductive modes: Thelytokous – Apex, Faison, Jackson Springs; Arrhenotokous – Mills River.*

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rarely reported in the literature, and nothing is known about the abundance and distribution of polyplودs. Although this analysis does not suggest that genetic structuring occurs as a result of polyplودdy in the NC populations sampled, tetraploidy was almost twice as common in these populations, and may be contributing to the establishment of clonal groups in some areas. Specific effects of polyplودdy have been reviewed recently in a series of publications ([46–52], and references therein), and include discussions on the production and maintenance of polyplودdids, genomic duplication and reduction events, genomic structuring, gene expression, gene dosage, changes in physiology, development, and fitness, breeding structure, genetic diversity, heterozygosity and inbreeding. More research is needed to address how polyplodium influences these traits in T. tabaci, especially with regard to the origin and presence of polyplodium, the relative distributions of diploid and polyplodium individuals, and how polyplodium influences population structure in arthropodous populations.

Another important area of research will be whether or not elevated gene copies are maintained and expressed, especially in relation to traits that may influence acquisition, infection and transmission of viruses, or genes related to insecticide metabolism or target sites whose elevated expression has been related to insecticide resistance in hemipteran and dipteran species [53].

The premise of this study to relate T. tabaci population structure to transmission efficiency of TSWV was born from observations that thrips and TSWV isolate, when grouped by location, were both significant explanatory variables in accounting for variation in the probability of TSWV transmission, and that, on average, transmission rates were higher among sympatric TSWV isolate-thrips pairings [22]. Investigating the probability of TSWV transmission by T. tabaci in relation to a specific ‘clonal assignment’ variable that uses the genetics of individual thrips, rather than using general population definitions such as ‘isofemale line’ that groups thrips transmission phenotypes based on where they were collected, revealed different, yet related, thrips tospovirus interactions. These interactions offer insights into the localized importance of this species as a vector of TSWV and highlight the role of genetic variation in isolate-specific vector competency among individual thrips within and among populations of T. tabaci in determining this species role in the epidemiology of TSWV over a broad range of spatial dimensions. While local adaptation between TSWV and T. tabaci, suggested by the significance of sympatry in earlier models, contributes to observed variation in vector competency among T. tabaci populations, ultimately, the interaction of specific thrips genotypes with specific virus isolates underlies transmission of TSWV by T. tabaci. Therefore, when assessing the importance of T. tabaci as a vector of TSWV, efficient transmission more likely depends on specific genotypic interactions between thrips clonal groups and virus isolates than on cryptic species group alone. However, cryptic species group, reproductive mode, and other life history traits affecting the fitness of these individuals will influence the abundance and distribution of efficient vectors through time and space. A better understanding of thrips population biology and defining the abundance and distribution of cryptic species and clonal groups over time would augment our understanding of thrips population dynamics and help to explain patterns of intra-and inter-population variation of economically important traits including vector competency.

Author Contributions
Conceived and designed the experiments: ALJ. Performed the experiments: ALJ WB. Analyzed the data: ALJ WB. Contributed reagents/materials/analysis tools: GGK ELV. Wrote the paper: ALJ WB GGK ELV.

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