

Comparative study of *Thrips tabaci* (Lindeman) cytochrome-c oxidase gene subunit I (COI) sequences data

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Vergleichende Studie von Sequenzdaten der Cytochrome-c-Oxidase Untereinheit I (COI) bei *Thrips tabaci* (LINDEMAN)

1 Introduction

The onion thrips (*Thrips tabaci* Lindeman) is one of the most important insect pests of onions and cabbages, but it also feeds on a wide variety of field crops including but not limited to: tobacco, cucumber, tomato and pepper (SHELTON et al., 1982, OROSZ et al., 2008, DIAZ-MONTANO et al., 2011). The onion thrips was the first recognised vector of the tomato spotted wilt virus (PITTMAN, 1927). According to Zawirska (1976), there are two subspecies of *T. tabaci*: members of the group *Thrips tabaci* 'communis' are only females, therefore, reproduction is restricted to parthenogenesis and they feed on a wide variety of crops. In con-

trast, members of the subspecies *Thrips tabaci* 'tabaci' only feed on tobacco, and are capable of sexual reproduction, but without copulation, all the offspring are males. After mating the offspring are either males or females (LEWIS, 1973). Based on genetic analysis, *T. tabaci* has been proposed to form a cryptic species complex. Using RAPD-PCR analysis, KLEIN and GAFNI (1996) found an intraspecific molecular variability among *T. tabaci* samples collected from three onion fields. Also, JENSER et al. (2001) documented genetic diversity with RAPD-PCR method between populations of *T. tabaci* collected from tobacco and onion. There is no external morphological difference between the adults of the two subspecies (ZAWIRSKA, 1976), but based on mi-

Zusammenfassung

Der Zwiebelthrips (*Thrips tabaci* Lindeman) ist eines der bedeutendsten Schadinsekten an einer Vielzahl von Kulturpflanzen. *T. tabaci* ist ein Komplex von kryptischen Arten, die Adulten sind anhand morphologischer Merkmale nicht zu unterscheiden. Eine Untersuchung der mitochondrialen DNS (wie der Cytochrome-c-Oxidase Untereinheit I (COI)) macht eine Unterscheidung möglich. Basierend auf ihren DNS-Sequenzen konnten drei Linien von *T. tabaci* nachgewiesen werden. Diese phylogenetischen Gruppen sind: ein mit Tabak assoziierter arrhenotoker Typus, ein mit Lauch assoziierter arrhenotoker Typus und ein thelytoker, mit Lauch assoziierter Typus. Im Rahmen dieser Studie wurden alle derzeit in der Datenbank des National Center for Biotechnology Information (NCBI) verfügbaren COI Gensequenzen des *T. tabaci* mit den Daten der vorliegenden Arbeit verglichen.

Schlagwörter: *Thrips tabaci*, COI-Sequenzen, Phylogenie.

Summary

The onion thrips (*Thrips tabaci* Lindeman) are one of the most important insect pests of a wide range of crops. *T. tabaci* is a cryptic species complex; the adults are undistinguishable based on morphological characters. The examination of mitochondrial DNA sequences (like the cytochrome-c oxidase subunit I, COI) makes the distinguishing possible. Based on mitochondrial DNA-sequences, three lineages of *T. tabaci* have been established. These phylogenetic groups are: arrhenotokous tobacco-associated type, arrhenotokous leek-associated type and thelytokous leek-associated type. This study aims to review all the currently available *T. tabaci* COI gene sequences in the National Center for Biotechnology Information (NCBI) database and compare it with our data.

Key words: *Thrips tabaci*, COI sequences, phylogeny.

tochondrial DNA-sequences, three lineages of *T. tabaci* have been established (BRUNNER et al., 2004). The three groups are the so-called L1, L2 (leek-associated) lineages and T (tobacco-associated) lineage. Later on, TODA and MURAI (2007) proposed that the reproductive mode of L1 and L2 was arrhenotoky and thelytoky, respectively. According to the above mentioned studies, the three known phylogenetic groups of *T. tabaci* are: arrhenotokous tobacco-associated type, arrhenotokous leek-associated type and thelytokous leek-associated type. To discriminate the two different reproductive modes TAKEUCHI and TODA (2011) suggested the use of a PCR-RFLP method but Kobayashi and Hasegawa (2012) established a simple method (PCR-SSP) they claimed would work well on most populations.

In the database of GenBank, the number of available *T. tabaci* mitochondrial DNA-sequences is continuously increasing.

We aim to review all the currently available *T. tabaci* COI gene sequences from the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) database and compare them with our samples.

2 Materials and methods

2.1 Thrips samples

The *T. tabaci* samples used in the comparative analysis were collected from different host plants in Hungary (Table 1). The Cab_TH1 sample derived from a thelytokous population, consisting of females only appeared for several generations. Thelytokous thrips were reared on cabbage leaf (*Brassica oleracea* L.) under laboratory conditions (16L:

8D, 23 °C). Allium_Ar 1, Tob_Ar1 and Tob_Ar2 samples were assumed to be arrhenotokous because of the presence of males in the population. Allium_Ar2 was a confirmed arrhenotokous line; offspring of the virgin females were all males. The uploading of the sequences to the NCBI database is in progress.

2.2 DNA extraction and PCR amplification

Total genomic DNA was extracted from a single adult thrips based on the method of Kikkert et al. (2006). To amplify the cytochrome-c oxidase subunit I (COI) region of the mitochondrial DNA, we designed specific sense (5'-GTAGT-GAAAGTGAGCTACAAC-3') and antisense (5'-CGAT-TAAATAATATAAGATTCTGACTWTTACC-3') primers based on the *T. tabaci* COI gene sequences from the NCBI database.

2.3 Phylogenetic analysis

More than 200 COI gene sequences are available in the NCBI database. In the highlighted comparative analysis, the sequences were different in size (from 629 to 810 base pairs), therefore only a 345 bp region, the common sections were compared. The UPGMA (Jukes-Cantor) method was used for constructing the phylogenetic tree with high bootstrap values, with the CLC Sequences Viewer programme. GenBank accession number and reproductive mode (if known) are included for each entry in the tree. Thrips imaginis was included as an outgroup species.

3 Results

The 780 bp region of the mitochondrial COI gene was successfully amplified.

As expected, the comparison of all available *T. tabaci* COI gene sequences resulted in three distinct groups (not shown). We presented only those sequences in our analyses, where information about the mode of reproduction was available (male sample or reproductive mode confirmed by sex ratio in virgin progeny) (Figure 1). The common section was a 345 bp region.

The first group, which is called TH, consists of the all known thelytokous strains and the specimen Cab_Th1, which was collected from cabbage in Hungary. In addition,

Table 1: Details of the collected samples (all sampled individuals are females)

Tabelle 1: Details zu den gesammelten Proben (alle gesammelten Individuen sind weiblich)

Sample name	Sequence length (bp)	Host plant	Reproductive mode
Cab Th1	780	Brassica oleracea	thelytokous
Allium Ar1	780	Allium cepa	arrhenotokous
Allium Ar2	780	Allium cepa	arrhenotokous
Tob Ar1	780	Nicotinia tabacum	arrhenotokous
Tob Ar2	780	Nicotinia tabacum	arrhenotokous

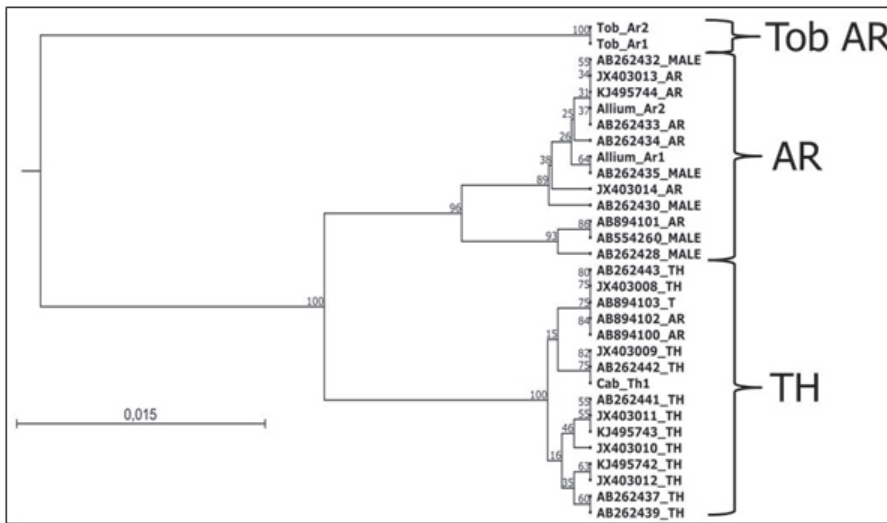


Figure 1: Phylogenetic tree of *Thrips tabaci* COI gene region
 Abbildung 1: Stammbaum der *Thrips tabaci* COI-Genregion

TH: Thelytokous, AR: Arrhenotokous, Tob AR :Tobacco associated arrhenotokous

two arrhenotokous individuals are found in this clade. SOGO et al. (2014) found that two virgin arrhenotokous females (Genbank AB894100 and AB894102) fell within the thelytokous group. They mentioned these individuals to “new arrhenotokous”.

The second group, which is named AR, consists of all known arrhenotokous strains and male specimens. Allium_Ar1 and Allium_Ar2 individuals, collected from onion in Hungary, belonged to this group too.

The third group, which is called Tob AR, consists of tobacco (*Nicotinia* spp.) associated specimens. The Tob_Ar1 and Tob_Ar2 samples, which were collected from tobacco in Hungary, belonged to this group.

The nucleotide sequences were translated to protein sequences. In addition, we compared our sequences with the

sequences of the same region from the database (Genbank AB262428-AB262444). These sequences particularly included the whole region of our samples.

Since the amplified regions are fragments of the COI gene, they do not represent the whole protein. Based on the examined region (1–780 bp), and considering their amino acid sequences, all the substitutions were silent, except in six samples that were different from the rest.

The AB262442-43-44 and Cab_Th1 were different from the rest of the samples: in the position of the 149th amino acid in the examined protein sequences there is a basic arginine (R) instead of a hydrophilic serine (S) (Figure 2). The nucleic acid AB262438 codes serine (S) instead of threonine (T) in the position of 53rd amino acid and the nu-

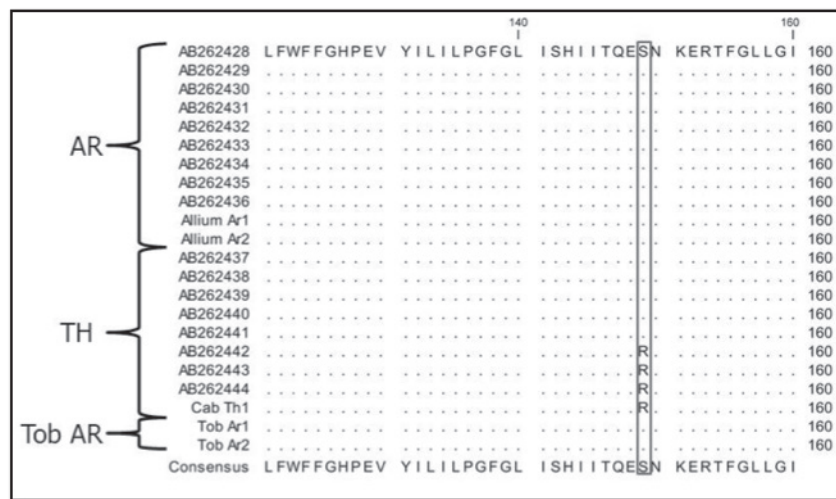


Figure 2: Multi-align of amino acid sequences of *T. tabaci* COI gene region
 Abbildung 2: Multi-align der Aminosäuresequenzen von *T. tabaci* COI Genregion

cleic acid AB262431 codes valine (V) instead of alanine (A) in the position of 79th amino acid.

4 Discussion

In the comparative study the 5 samples collected in Hungary were placed in three different groups of the phylogenetic tree, and they meant at least one new haplotype contribution to each group. All the available nucleotide sequences of the NCBI database were placed in three groups, but the reproductive mode is not determined for most of the samples, thus it is still unclear, if the establishment of these groups coincides with differences in host fidelity and reproductive mode of the specimens. SOGO et al. (2014) suggested that the methods currently used to distinguish thelytokous and arrhenotokous individuals (TAKEUCHI and TODA 2011, KOBAYASHI and HASEGAWA 2012) need to be revised. The DNA sequences of these two “new arrhenotokous” (Genbank AB894100 and AB894102) specimens, which have the same characteristic as thelytokous mitochondrial COI haplotype, were found in field populations in Japan (SOGO et al., 2014). It is assumed that these rare arrhenotokous individuals were produced by successful mating between thelytokous females and arrhenotokous males. This hypothesis could explain why they reproduced by arrhenotoky and carried maternally inherited thelytokous mitochondrial COI haplotype. Based on this assumption, this record is the first report about gene-transfer between two different biotypes of *T. tabaci* in a sympatric field population. In a recent study LI et al. (2015) showed that mating and gene transfer from arrhenotokous *T. tabaci* to thelytokous *T. tabaci* is possible in a laboratory population. For clarification of this hypotheses, further studies about *T. tabaci* mating behaviour are needed.

Our future goal is to establish clean laboratory cultures of each lineages of *T. tabaci*, and to develop a reliable and rapid method for the identification of these three different types. This method would also be helpful for further studies involving different *T. tabaci* biotypes.

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