Identification of thrips using ITS-RFLP analysis

Moritz, G., Paulsen, M., Delker, C., Picl, S. and Kumm, S.
Institute of Zoology, Martin-Luther-University Halle-Wittenberg, Domplatz 4, 06099 Halle/Saale, Germany.
E-mail: moritz@zoologie.uni-halle.de

Introduction

Among the 5500 thrips species described worldwide only one per cent are known as pest species and only about ten species have been confirmed as vectors of plant viruses (Lewis, 1997). Because of their rapid and parthenogenetic reproduction and their feeding behaviour, pest thrips can cause considerable damage. Identification of Thysanoptera is often difficult, and no useful identification key exists for preadult stages based on morphological characters. The use of restriction fragment length polymorphism (RFLP) of the internal transcribed spacer (ITS) region of the rDNA has the advantage of combining highly conserved sequences in the 18S, 28S, and 5.8S rDNA regions with variable sequences in the ITS regions at species level (Moritz et al., 2000). Moreover, the ITS shows a high interspecific variability with extremely low intraspecific variability.

Materials and Methods

The following thrips species were investigated: *Echinothrips americanus* (Ea), *Frankliniella occidentalis* (Fo), *Frankliniella tenuicornis* (Ft), *Helionothrips aino* (Ha), *Helionothrips spinosus* (Hs), *Heliothrips haemorrhoidalis* (Hh), *Hercinothrips femoralis* (Hf), *Limothrips cerealium* (Lc), *Limothrips denticornis* (Ld), *Moundothrips apterygus* (Ma), *Parthenothrips dracaenae* (Pd), *Pseudanaphothrips achaetus* (Pa), *Rhipiphorothrips cruentatus* (Rc), *Selenothrips rubrocinctus* (Sr), *Sigmothrips aotearoana* (Sa), *Suocerathrips linguis* (Sl), *Thrips nigropilosus* (Tn), *Thrips physapus* (Tp), and *Thrips tabaci* (Tt).

DNA-extraction

DNA was extracted from all developmental stages (modified after (Roberts, 1998)). A single specimen was ground with a micro pestle in a sterile 1.5ml Eppendorf tube while adding 100µl of extraction buffer (0.2M Sucrose, 0.1M Tris, 0.1M NaCl, 0.05M EDTA, 0.5 % SDS, pH 9.2). The tubes were heated at 65°C for 15-30 minutes. After adding 15µl of cold 8M potassium acetate and incubating on ice for 15 minutes the tubes were centrifugated 20 minutes at 10000x g. The supernatant was transferred to a 1.5ml tube and the same amount isopropanol was added followed by spinning at 10000x g for 15 minutes. The pellet was washed twice with 70 % ethanol, dried, and re-suspended in 16µl sterile bi-distilled water.

PCR-RFLP

The ITS2 was amplified using the primers 28Z 5´AGACTCCTTGGTCCGTGTTTC 3´ (Hillis and Dixon, 1991) and P1 5´ATCACTCGGCTCGTGGATCG 3´ (Severini et al., 1996). The polymerase chain reaction mixture contained 1x PCR-buffer, 100mM (0.2mM) each of dATP, dCTP, dGTP, dTTP, 2mM $MgCl_2$, 0.6M (0.15 µM) of each primer, 1 unit Taq-polymerase (Eppendorf), and sterile bi-distilled water to a final volume of 50µl. The amplification was carried out in an Eppendorf mastercycler gradient. The DNA was initially denatured at 95°C for 3 minutes followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 57°C for 45 seconds, and elongation at 72°C for 2 minutes. The last cycle was followed by a 4 minutes incubation period at 72°C to complete any partially synthesized strands. Amplification was followed by the RFLP. 7µl of the PCR-products were digested with 5 units of the enzymes *Rsa*I, *Hae*III, *Msp*I, *Hin*fI, and *Alu*I. Restriction fragments were separated by gel electrophoresis in a 2% agarose gel stained with ethidium bromide and analyzed with an ImageMaster VDS (Pharmacia Biotech) using the software ImageMaster 1D Elite v 3.01.

Cloning and sequence analysis

In addition to the restriction a second PCR product was cloned into the plasmid pGem-T (Promega) and transformed into JM109 high-competent cells. Double-stranded sequencing was performed using dRhodamine Terminator Cycle Sequencing Kit and sequencing primers Sp6 and T7 for pGem-T (Promega). Amplification was carried out in a Biometra Trio Thermoblock. Sequence data were aligned using the programs GCG/Seqlab Version 10.0 and Clustal W (1.8). PhyloWin (Version 1.2) was used for creating the most parsimony and likelihood tree. DNA sequences can be found in the EMBL/GenBank (accession number: AJ303091, AJ308591, AJ308592, AJ308593, AJ308594, AJ308595 and AJ308596).

Results and Discussion

All species show a typical size of their PCR products (amplified ITS2 region: table 1). The patterns of the RFLP analysis were characteristic for each species. In a few cases we found nearly similar patterns of one restriction enzyme in several species, but this was compensated by the use of five different restriction enzymes. Figure 1 shows the RFLP of *Thrips tabaci*. Because ITS sequences are DNA pieces with no coding function we used the sequenced data of the ITS2, the 28S and the 5.8S fragments for a similarity, and not a phylogenetic analysis. As expected the randomised regions correlate with the genus level (Fig. 2).

Acknowledgements

We thank Laurence Mound, Antoon Loomans, G. McLaren, Tamotsu Murai, Shuji Okajima, Irene Terry, Bert Vierbergen, and Chao Wang for providing thrips samples, and Laurence Mound for discussion and critical comments.

Species	bp
Thrips physapus	1322
Thrips tabaci	1331
Frankliniella occidentalis	1351
Frankliniella tenuicornis	1373
Pseudanaphothrips achaetus	1379
Heliothrips haemorrhoidalis	1403
Rhipiphorothrips cruentatus	1407
Helionothrips aino	1431
Helionothrips spinosus	1449
Sigmothrips aotearoana	1487
Echinothrips americanus	1492
Limothrips cerealium	1516
Limothrips denticornis	1542
Parthenothrips dracaenae	1598
Hercinothrips femoralis	1620
Thrips nigropilosus	1679
Selenothrips rubrocinctus	1796
Moundothrips apterygus	1804
Suocerathrips linguis	2433

Table 1. Average size of ITS2 fragment

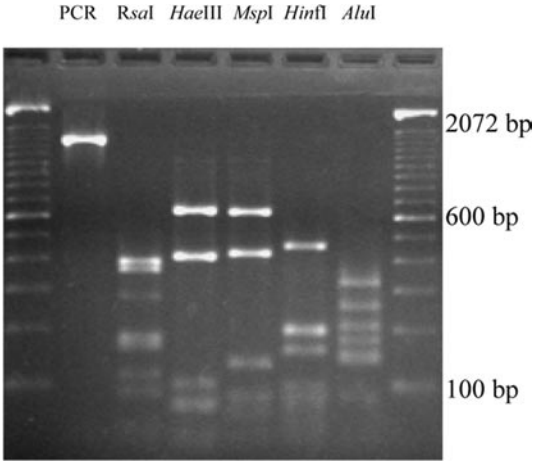

Figure 1. RFLP pattern of *Thrips tabaci*

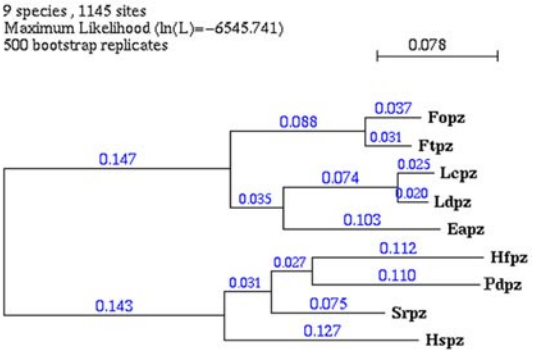

Fig. 2: Cluster analysis using PhyloWin (pz = Primer P1/28Z)

References

Hillis DM and Dixon T. 1991. Ribosomal DNA: Molecular evolution and phylogenetic interference. *The Quarterly Review of Biology* **66**, 411-453.

Lewis T. 1997. *Thrips as crop pests*. CAB International Oxford and New York.

Moritz G, Delker C, Paulsen M, Mound LA and Burgermeister W. 2000. Modern methods for identification of Thysanoptera. *EPPO Bulletin* **30**(3/4), 591-593.

Roberts DB. 1998. *Drosophila: a practical approach*. University Press Oxford, Oxford.

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