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INTRA- AND INTERSPECIFIC MOLECULAR POLYMORPHISM OF THRIPS SPECIES

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Molecular polymorphism of six species of Thysanoptera of both sexes, collected from different locations and host plants in Hungary was studied by using RAPD-PCR technique. The specimens were classified according to sampling sites (Gödöllő, Nagykovácsi and Valkó), host plants *(Lathyrus tuberosus, Medicago sativa, Taraxacum officinale, Trifolium pratense),* sexes, and larvae in case of *Aeolothrips intermedius*. On the basis of the total of 103 fragments generated by 15 RAPD primers the genetic distances were calculated by cluster analysis using simple matching method. The dendrogram resulted in two main groups: Aeolothripidae *(Aeolothrips intermedius)* and Thripidae *(Frankliniella intonsa, Kakothrips robustus, Odontothrips confusus, Thrips dilatatus* and *T. tabaci)*. Within the family Thripidae two subgroups were observed including (i) *F. intonsa, T. dilatatus* and *T. tabaci,* and (ii) *K. robustus* and *O. confusus*. Two population-specific and one sex-linked fragments were identified by the RAPD primers, OPQ14, NO11 and OPA08, respectively.

Keywords: Molecular polymorphism – RAPD – Aeolothripidae – Thripidae – Thysanoptera

INTRODUCTION

As all insects, Thysanoptera species are classified on the bases of morphological characters of systematics. This type of classification has been raising problems and directed the attention to the significance of molecular taxonomy, since morphologically identified individuals belonging to the same species may differ from each other in such an important trait as like virus transmission [14]. This is the main reason why several research groups have already applied RAPD-based investigations to classify thrips species. Cenis and Beitia [1] compared *Frankliniella occidentalis* with other thrips species of *Limothrips,* and *Aeolothrips*, and several Diptera and Homoptera detecting typical species-specific DNA-fingerprints. Klein and Gafni [5] used RAPD markers to discriminate three different morphological types of onion thrips, *T. tabaci*. Kraus et al. [6] also used RAPD-PCR to study intraspecific variability of *F. occidentalis* detecting a DNA fragment suitable to distinguish *F. occidentalis* populations

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originated from Switzerland and Australia. Even when classical systematics seemed to be satisfactory to distinguish populations and species, some observations and considerations raised doubts about the use of the morphological characters.

In the light of these problems we aimed to provide molecular markers which can supply new systematic characteristics for thrips taxonomy in cases of population- and sex-specific molecular alterations.

MATERIALS AND METHODS

Origin of thrips samples

Thrips were collected from their host plants, *Medicago sativa*, and *Trifolium pratense* (terminal shoots), *Taraxacum officinale*, and *Lathyrus tuberosus* (flowers). The sampling sites were: Nagykovácsi (latitude 47°31′N, longitude 18°50′N), Gödöllő (47°34′N, 19°21′N) and Valkó (47°30′N, 19°27′N) (Table 1). Thrips were identified and separated to sexes under stereomicroscope according to Jenser [4], and Mound and Kibby [8].

Table 1
The sampling sites of thrips populations, host plants and sexes of thrips species studied

No.	Species	Sampling site	Host plant	Sex
1	*Frankliniella intonsa*	Nagykovácsi	*Medicago sativa*	female
2	*F. intonsa*	Nagykovácsi	*M. sativa*	male
3	*F. intonsa*	Valkó	*Trifolium pratense*	female
4	*F. intonsa*	Valkó	*M. sativa*	male
5	*F. intonsa*	Valkó	*M. sativa*	female
6	*Kakothrips robustus*	Gödöllő	*Lathyrus tuberosus*	female
7	*K. robustus*	Gödöllő	*L. tuberosus*	male
8	*Aeolothrips intermedius*	Valkó	*M. sativa*	female
9	*Ae. intermedius*	Valkó	*M. sativa*	male
10	*Ae. intermedius*	Valkó	*T. pratense*	female
11	*Ae. intermedius*	Nagykovácsi	*M. sativa*	male
12	*Ae. intermedius*	Nagykovácsi	*M. sativa*	female
13	Larvae of *Ae. intermedius*	Nagykovácsi	*M. sativa*	female and male
14	*Odontothrips confusus*	Nagykovácsi	*M. sativa*	female
15	*O. confusus*	Nagykovácsi	*M. sativa*	male
16	*Thrips tabaci**	Valkó	*M. sativa*	female
17	*T. tabaci**	Nagykovácsi	*M. sativa*	female
18	*Thrips dilatatus*	Valkó	*Taraxacum officinale*	female
19	*T. dilatatus*	Valkó	*T. officinale*	male

* Parthenogenesis occurs in *T. tabaci,* in Hungary (Jenser, G., personal communication).

DNA extraction

For DNA extraction 100 individuals were collected into 19 pools, which were formed according to species, populations of sampling sites, host plants and sexes (Table 1). Until DNA isolation, thrips pools were stored in Eppendorf tubes containing 1 ml of DNA extraction buffer of 1% hexadecyltrimethylammonium bromide (CTAB, Sigma) at –20 °C. The extraction was performed according to the CTAB procedure [2] as modified by Mendel et al. [7]. Thrips were ground to fine powder under liquid nitrogen. 400 µl of DNA extraction buffer at 60 °C was added to each tube. The homogenate was incubated at 60 °C for 30 min, followed by an addition of equal volume of chloroform:isoamyl alcohol (24 : 1) and separated by a microcentrifuge at 13,000 rpm for 1 min. Total DNA was precipitated with isopropanol to the aqueous phase, washed in 70% ethanol, dried and resuspended in 100 µl $T_{10}E_1$ buffer (10 mM Tris, 1 mM EDTA). The quality of isolated DNA was determined on a 0.8% agarose gel stained with ethidium bromide.

PCR amplification

For RAPD analysis 15 decamer primers were applied (Table 2). PCR amplification was carried out in 25 µl reaction mixture containing 10–20 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 2 mM $MgCl_2$, 0.75 µM dNTPs, 60 nM primer, 1.5 U Taq DNA polymerase (Promega). Cycling was carried out in a Perkin–Elmer 9600 thermocycler: 1 cycle of 94 °C for 2 min; 40 cycles of these steps of 94 °C for 10 sec, 36 °C for 30 sec, 72 °C for 1 min, and closed by 1 cycle of 72 °C for 2 min. Electrophoresis was performed in 1.2% agarose (FMC) gels. Fragment sizes were estimated by comparison to DNA molecular weight markers (Boehringer Mannheim, Fermentas).

Analysis of PCR amplification profiles

PCR amplification bands were scored as present (1) or absent (0). The similarity was analysed on the basis of the number of shared amplification products according to simple matching method. A dendrogram based on similarity coefficient indices was generated with the program Syn-Tax-pc 5.0 by means of the unweighted pair group method of arithmetic means (UPGMA) [10].

RESULTS

All fifteen RAPD primers tested produced scorable PCR-band pattern in all the thrips pools. The number of bands varied from 2 to 15 per primer and the sizes of the bands varied from several of hundreds basepairs (bp) up to 3000 bp. Most primers unambiguously differentiated the thrips species resulting in species-specific bands (Fig. 1).

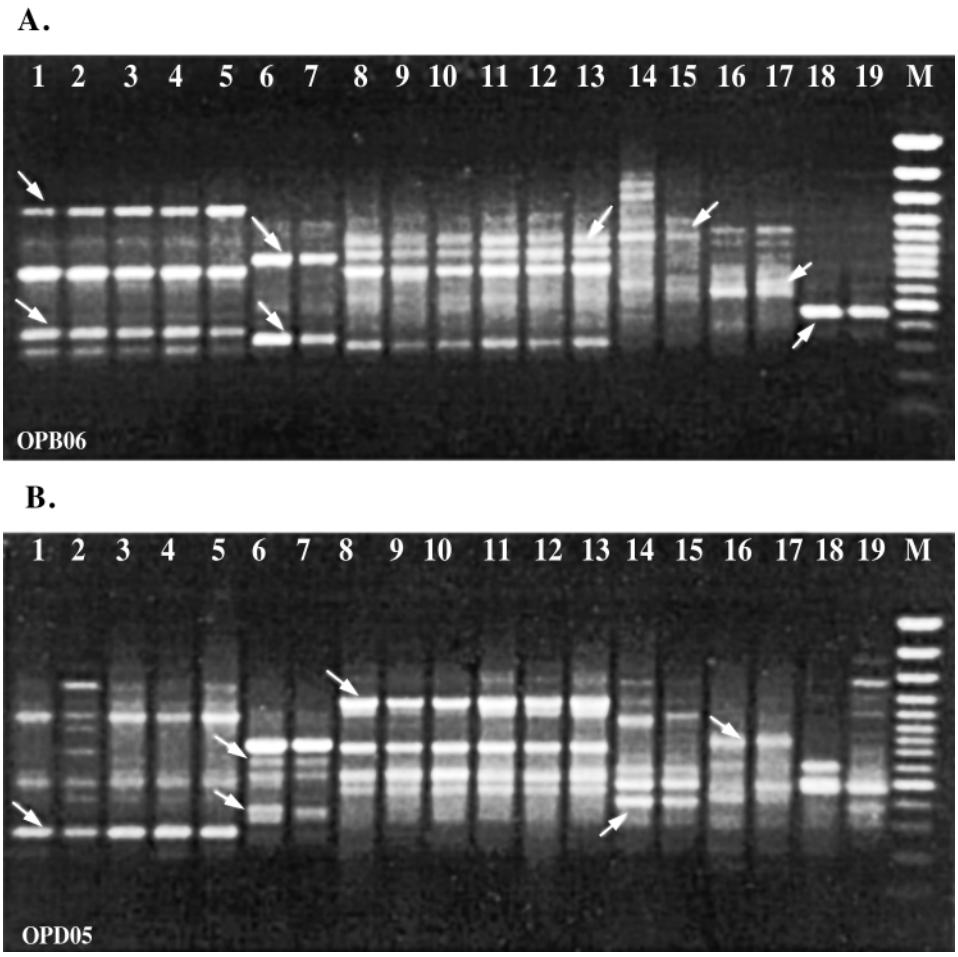

Fig. 1. Amplification patterns generated by primers of OPB06 (A) and OPD05 (B). Lanes 1–5: *F. intonsa;* Lanes 6–7: *K. robustus;* Lanes 8–13: *Ae. intermedius;* Lanes 14–15: *O. confusus;* Lanes 16–17: *T. tabaci;* Lanes 18–19: *T. dilatatus;* M (Mw): 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp in length). Arrows indicate species-specific fragments

Monomorphic band patterns among species were not obtained at all, and there were only a few fragments appearing in more species at the same time.

The cluster analysis of 103 characteristic bands of 15 primers clustered two main groups according to the families of Aeolothripidae and Thripidae (Fig. 2). As expected, *Ae. intermedius*, the only species of Aeolothripidae, differed from all the remaining species characteristically. Within the family Thripidae there were two well-defined subgroups observed: (i) *F. intonsa, T. dilatatus* and *T. tabaci* and (ii) *K. robustus* and *O. confusus*. Unexpectedly, the dendrogram clustered the two *F. intonsa* populations of Nagykovácsi (Nos 1, 2) and Valkó (Nos 3, 4, 5) separately, as the

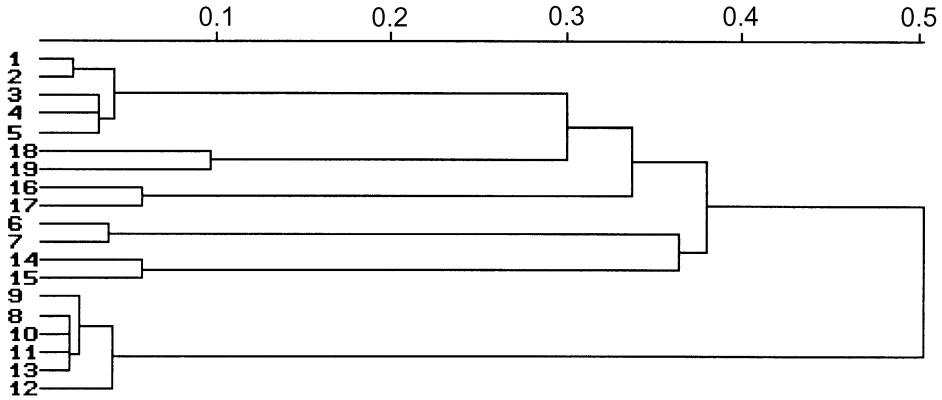

Fig. 2. Dendrogram of 19 thrips pools based on RAPD analysis produced by the Syn-Tax-pc cluster analysis program. The scale is based on simple matching coefficient of similarity. Pools 1–5: *F. intonsa;* Pools 6–7: *K. robustus;* Pools 8–13: *Ae. intermedius;* Pools 14–15: *O. confusus;* Pools 16–17: *T. tabaci;* and Pools 18–19: *T. dilatatus*

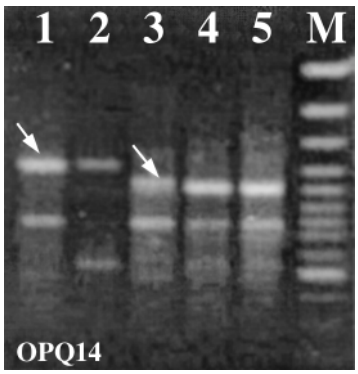

Fig. 3. Molecular polymorphism among populations of *Frankliniella intonsa.* Gel electrophoresis after PCR amplification with primer OPQ14. Mw: 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp in length. Populations of Nagykovácsi (1 to 2), and Valkó (3 to 5). Arrows indicate the population-specific fragments

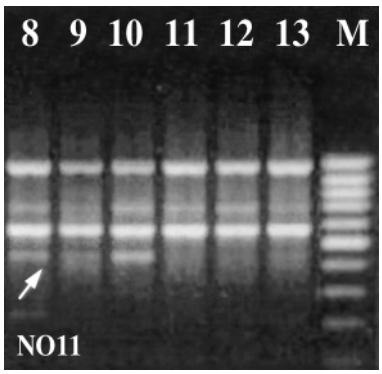

Fig. 4. Molecular polymorphism among populations of *Aeolothrips intermedius*. Gel electrophoresis after PCR amplification with primer NO11. Mw: 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100, 80 bp in length. Populations of Valkó (8 to 10) and Nagykovácsi (11 to 13). Arrow indicates the population-specific fragments

results of the primer reaction of OPQ14 which amplified a fragment (~750 bp) in the *F. intonsa* pools (Nos 1, 2) sampled at Nagykovácsi was missing from the samples of Valkó. At the same time a PCR-fragment (~650 bp) was present in the Valkó pools, whereas it was missing from the samples of Nagykovácsi (Fig. 3). For *Ae. inter-*

medius the primer NO11 differentiated a fragment (~450 bp) in the Valkó samples (No. 8 to 10) which was absent from the samples of Nagykovácsi (No. 11 to 12) (Fig. 4).

No molecular differences were obtained among the populations of *F. intonsa* and *Ae. intermedius* collected from different host plants, *M. sativa* and *T. pratense* at the same location of Valkó. For example the samples of Nos 3 and 10 originating from *T. pratense* (Valkó) did not differ from the samples of Nos 1, 2, 4, 5 and 8, 9, 11, 12, 13 taken from the flowers of *M. sativa* (Valkó) (Tables 1 and 3).

In the case of *Ae. intermedius* a fragment of about 800 bp appeared only in females (Nos 8, 10, 12) and larvae (No. 13) but not in males (Fig. 5). Obviously, the sample of larvae No. 13 was a mixed pool of both sexes because the separation at species level proved to be difficult, as no keys were available for identifying the sexes.

Table 2
RAPD primers and sequences used

Primer	Sequence (5′-3′)	Primer	Sequence (5′-3′)	Primer	Sequence (5′-3′)
OPA01	CAGGCCCTTC	OPD05	TGAGCGGACA	OPAB09	GGGCGACTAC
OPA07	GAAACGGGTG	OPJ09	TGAGCCTCAC	NO08	ATCCGCGTTC
OPA08	GTGACGTAGG	OPQ14	GGACGCTTCA	NO11	ACGGCATATG
OPB06	TGCTCTGCCC	OPX11	GGAGCCTCAG	UBC354	CTAGAGGCCG
OPC15	GACGGATCAG	OPAI21	CACGCGAACC	PAL2	CCAGGTGGACC

Table 3
Primers resulting in distinctive RAPD fragments for species, populations and sexes of *Thysanoptera*

Trait	Primer	Species	Fragment size (bp)
Species-specific	OPB06	*F. intonsa*	~1300, ~400
		K. robustus	~900, ~350
		Ae. intermedius	~950
		O. confusus	~1050
		T. tabaci	~1100
		T. dilatatus	~480
	OPD05	*K. robustus*	~700, ~400
		Ae. intermedius	~1200, ~300
		O. confusus	~450
		T. tabaci	~800
Sex-linked	OPA08	*Ae. intermedius*	>800[1]
Population-specific	NO11	*Ae. intermedius*	~450[2]
	OPQ14	*F. intonsa*	~750, ~650[3]

[1] Sex-linked fragment in females and both sexes of larvae.

[2] Population-specific fragment in the Valkó pools of *Ae. intermedius.*

[3] Population-specific fragments in *F. intonsa.* One ~750 bp fragment was present in the pools of Nagykovácsi, another ~650 bp in the pools of Valkó.

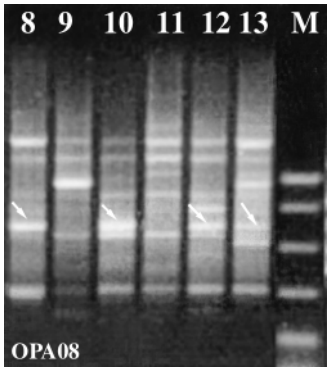

Fig. 5. Sex-linked molecular polymorphism in *Aeolothrips intermedius.* Gel electrophoresis after PCR amplification with primer OPA08. Mw: 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100 and 80 bp in length. Populations of Valkó (8 to 10) and Nagykovácsi (11 to 13). Arrows indicate the sex-linked fragments

DISCUSSION

In Thripidae species-specific distinctive PCR-fragments were already detected as in the comparison of *F. occidentalis* and *T. tabaci* [6]. Perring et al. [9] successfully separated the morphologically indistinguishable silver-leaf type of *Bemisia tabaci* from the sweet-potato type by using molecular markers.

In the present study a total of 103 fragments of the 15 RAPD primers were found that prove genetic distances of the various species of Thysanoptera as RAPD patterns. The resulted two main groups of Aeolothripidae *(Ae. intermedius)* and Thripidae *(F. intonsa*, *K. robustus*, *O. confusus, T. dilatatus* and *T. tabaci)* were identical to the systematic classification. Unexpectedly, population-specific markers were also identified in the two populations of *F. intonsa* (Nos 1–2 and 3 to 5) by the primers OPQ14, and NO11. These molecular differences between the two populations of *F. intonsa* living on the same host plants *(M. sativa* and *T. pratense)* seemed to prove an intraspecific genetic difference which could be caused by varying allele-frequencies at sub-population level as a result of the different ecological circumstances. Similarly to our results, Kraus et al. [6] using the primer OPA07 exhibited a distinct DNA fragment in *F. occidentalis* populations derived from different locations of Switzerland and Australia.

We also detected a sex linked PCR-fragment in *Ae. intermedius* with the primer OPA08. The PCR-fragment of about 800 bp was present in the pools of larvae and females but was absent in the pool of males. Of the three main types of parthenogenetic species as like arrhenothoky, thelytoky and deuterotoky, Thysanoptera belong to the arrhenotoky type which means haplodiploidy with males developing

from unfertilised eggs (X0) while females develop from fertilised eggs (XX) [3, 11, 12, 13]. On the basis of this sex characteristic the extra polymorphic DNA band in the pools of female and larvae can only be explained by a doubled copy number of sex linked X-chromosome.

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