Genetic variation within and among populations of *Aeolothrips intermedius*

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Abstract: Genetic variations within and among populations of *Aeolothrips intermedius* Bagnall were analysed using RAPD-PCR. Adults and larvae of this species were collected from lucerne (*Medicago sativa*) and red clover (*Trifolium pratense*) at two locations in Hungary, Nagykovácsi and Valkó. Intraspecific molecular polymorphism, including both population-specific and female-linked PCR-fragments, was identified by RAPD primers of NO11 and OP-A-08, respectively.

Keywords: intraspecific polymorphism; RAPD; Aeolothripidae; Thysanoptera.

Introduction

Thysanoptera species are classified on the basis of morphological characters, but molecular tools will be needed to discriminate between conspecific populations differing in such important traits as virus transmission (Wijkamp et al. 1995). PCR-based techniques have already been applied to intra- and inter-specific polymorphism in Thysanoptera species (Cenis and Beitia 1994; Klein and Gafni 1996; Kraus et al. 1999), and suitable DNA fragments were detected to distinguish thrips populations originating from geographically different locations (Kraus et al. 1999). In this study we aimed to detect molecular diversity of two populations of *Aeolothrips intermedius* from lucerne and red clover.

Materials and Methods

Samples. Specimens of *Ae. intermedius* were collected from terminal shoots with flowers of *Medicago sativa* and *Trifolium pratense*. The sampling sites were: Nagykovácsi (latitude 47°31´N, longitude 18°50´N), and Valkó (47°30´N, 19°27´N). Identification was based on: Dyadechko (1964), Jenser (1982), and Mound and Kibby (1998).

DNA extraction. Each pool contained 100 individuals deriving from two populations (Table 1). Until DNA isolation, the pools were stored in Eppendorf tubes containing 1 ml of DNA extraction buffer of 1% hexadecyltrimet hylammonium bromide (CTAB, Sigma) at –20 °C. The extraction was performed according to the CTAB procedure (Doyle and Doyle 1990) as modified by Mendel et al. (1994). Thrips were ground to fine powder. 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 24 chloroform : 1 isoamyl alcohol and separated by a microcentrifuge at 13000

Nº	Species	Sampling site	Host plant	Sex
1	*Ae. intermedius*	Valkó	*Medicago sativa*	female
2	*Ae. intermedius*	Valkó	*M. sativa*	male
3	*Ae. intermedius*	Valkó	*T. pratense*	female
4	*Ae. intermedius*	Nagykovácsi	*M. sativa*	male
5	*Ae. intermedius*	Nagykovácsi	*M. sativa*	female
6	Larvae of *Ae. intermedius*	Nagykovácsi	*M. sativa*	female and male

Table 1. The sampling sites, host plants and sexes of *Aeolothrips intermedius* studied

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 2. RAPD primers and their sequences used

r.p.m. for 1 min. Total DNA was precipitated with isopropanol to the aqueous phase, washed in 70% ethanol, dried and re-suspended in 100 µml $T_{10}E_1$ 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 done 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 (GeneRuler™ 100bp Ladder, GeneRuler™ 100bp Ladder Plus, Fermentas).

Analysis of PCR amplification profiles. PCR amplification bands were scored as present (1) or absent (0).

Results

All the fifteen RAPD primers tested produced scorable PCR-band pattern. The number of bands varied from 2 to 15 per primer and the size of the bands varied from several hundred base pairs up to 1500 bp. For *Ae. intermedius* the primers of OP-D-05 and NO11 differentiated a polymorphic fragment at about 1000 bp in the Nagykovácsi population (Fig. 1/A) and at about 450 bp in the Valkó population (Fig.1/B). No molecular differences were obtained among the *Ae. intermedius* collected at Valkó (Fig. 1) from *M. sativa* (Nº 1-2) and *T. pratense* (Nº 3).

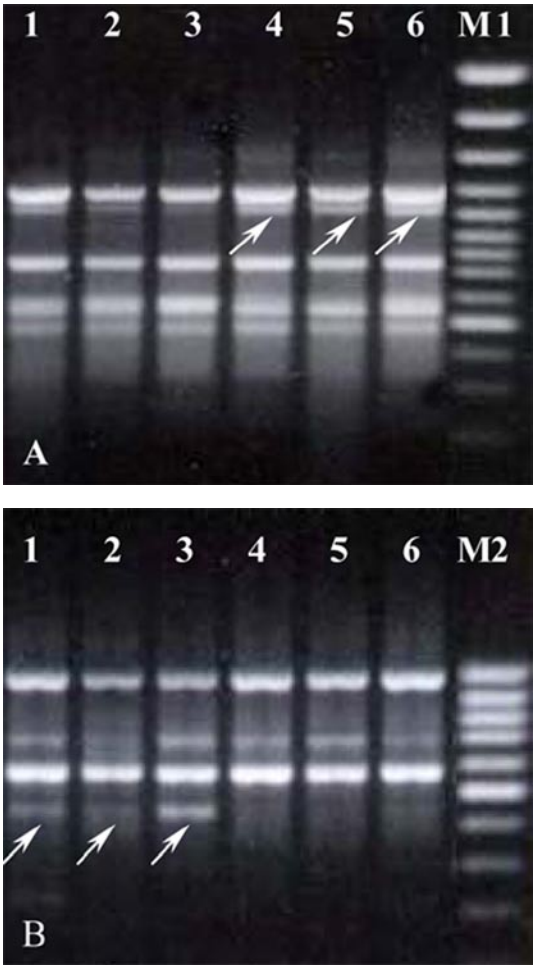

Figure 1. Molecular polymorphism among populations of *Aeolothrips intermedius*. Gel electrophoresis after PCR amplification with the primers of OP-D-05 (A) and NO11 (B).

Lanes 1-3: Valkó; Lanes 4-6: Nagykovácsi; M1 (Mw): 3000, 2000, 1500, 1200, 1031, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp in length; M2: 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100, 80 bp in length. Arrows indicate the population-specific fragments.

In populations at both Valkó and Nagykovácsi, a polymorphic PCR-fragment at about 800 bp appeared in females (N° 1, 3, 5) and larvae (N° 6), but not in males (N° 2 and 4) (Fig. 2). Obviously, the sample of larvae N° 6 was a mixed pool of both sexes. The final experiments whether this fragment can be considered as a sex-linked marker is under progress.

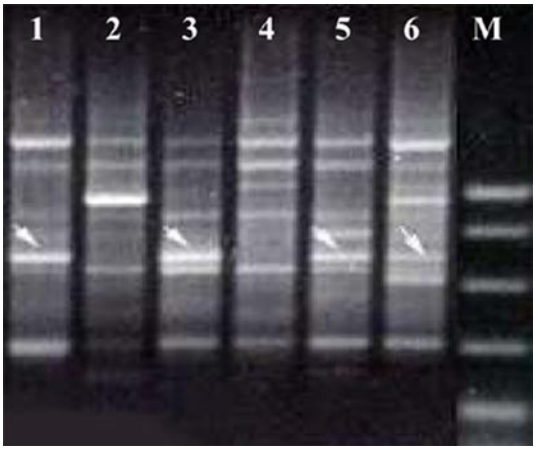

Figure 2. Female-linked molecular polymorphism in *Ae. intermedius*. Gel electrophoresis after PCR amplification with the primer of OP-A-08. M (Mw): 1031, 900, 800, 700, 600, 500, 400, 300, 200, 100, and 80 bp in length. Lanes 1, 3, 5-6: females and larvae; Lanes 2 and 4: males. Arrows indicate the female-linked fragments.

Discussion

In the Homopteran, *Bemisia tabaci*, Perring et al. (1993) successfully separated the morphologically indistinguishable silver-leaf type from the sweet-potato type using molecular markers. In Thripidae, species-specific distinctive PCR-fragments have been detected in *F. occidentalis* and *T. tabaci* (Kraus et al. 1999). In our study, we identified a population-specific marker by the primer NO11. This molecular difference between two populations was presumably caused by varying allele-frequencies at sub-population level as a result of differing ecological circumstances. Similarly, Kraus et al. (1999) using the primer of OP-A-07 found a DNA fragment difference between *F. occidentalis* populations from Switzerland and Australia.

We also detected a female-linked PCR-fragment in *Ae. intermedius* with the primer OP-A-08. The PCR-fragment of about 800 bp was present in the pools of larvae and females but was absent in the pool of males. Thysanoptera are arrhenotokous, with males developing from unfertilised eggs (X0) while females developing from fertilised eggs (XX) (Whiting 1945, Riesler and Kempter 1961, Hartl and Brown 1970, White 1973). This doubled copy of the X-chromosome may explain the extra polymorphic DNA band we found in the pools of females and larvae.

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