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PRIMER NOTE

Isolation and characterization of microsatellite loci in the avocado thrips *Scirtothrips perseae* (Thysanoptera: Thripidae)

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Abstract

The recent invasion of California by avocado thrips, *Scirtothrips perseae*, has had a serious economic impact on the Californian avocado industry. Here we report the isolation and characterization of six microsatellite loci for *S. perseae*, four of which were highly polymorphic (number of observed alleles ranged from three to 13 and expected heterozygosity from 0.31 to 0.87). These markers will be used to investigate the invasion history and route of entry into California of *S. perseae*. Three of the six loci successfully amplified in other *Scirtothrips* and *Neohydatothrips* species.

Keywords: avocado thrips, incursion management, insect crop pest, invasive species, microsatellites, *Scirtothrips perseae*

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Avocado thrips, *Scirtothrips perseae* Nakahara, is a serious exotic pest of avocado that was first discovered attacking avocados in California in 1996 and quickly spread to more than 95% of California's avocado acreage (Hoddle *et al*. 2002). The arrival of this pest has seriously impacted the Californian avocado industry, with long-term losses estimated to be around $4.45–$8.51 m per year (Hoddle *et al*. 2003). At the time of its discovery in California, *S. perseae* was new to science, but the home range of this insect has since been delineated as a region stretching from high altitude areas in the state of Michoacan, central Mexico, to central Guatemala (Hoddle *et al*. 2002). However, within this region, the distribution of this insect is fragmented and distinct populations are likely to exist. There is a need to further refine our knowledge of the origin of the Californian population of *S. perseae* in order to identify the routes of entry of this pest and to focus our search on natural enemies as part of a biological control initiative. Microsatellite markers are required to study population structure at an interpopulation scale. Furthermore, a recent taxonomic review of *Scirtothrips* in Mexico listed seven species from avocado, of which, five were previously undescribed (Johansen & Mojica-Guzman 1998). The validity of this review has been questioned (Mound & zur Strassen 2001), and microsatellite markers may help in determining the number of 'true' *Scirtothrips* species on avocado in Mexico. Here we report characteristics and primers for the first microsatellite loci isolated for *S. perseae*.

Total genomic DNA was extracted from 30 individuals (collected from several locations in California during 2002) using a phenol–chloroform extraction method with an RNase and proteinase-K treatment (see Sambrook & Russell 2001). Microsatellites were isolated using the fast isolation by AFLP (amplified fragment length polymorphism) of sequences containing repeats (FIASCO) protocol described by Zane *et al*. (2002). DNA was simultaneously digested with *Mse*I (New England BioLabs) and ligated to *Mse*I AFLP adaptors (5′-TACTCAGGACTCAT-3′/5′-GACGATGAGTCCTGAG-3′). The resulting digestion–ligation mixture was then directly amplified using AFLP adaptor-specific primers (5′-GATGAGTCCTGAGTAAN-3′) and the polymerase chain reaction (PCR). Following this 'preselective amplification', amplicons were enriched for repeat sequences by hybridization to biotinylated microsatellite oligonucleotides [$(AC)_{17}$, $(GA)_{11}$, $(AGG)_{11}$, $(ATT)_{11}$ and $(GATA)_9$] and selectively captured using magnetic streptavidin-coated beads. DNA

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Table 1 Characterization of six microsatellite loci from *Scirtothrips perseae*. Includes optimized PCR conditions [annealing temperature (T_a) and concentrations of $MgCl_2$ and DMSO] for each locus. Null alleles were present at Sper14 and Sper19. The clone sequences for these primer pairs correspond to GenBank Accession nos: AY885655–AY885660

Locus	Primer sequences (5′–3′)	T_a (°C)	$MgCl_2$	DMSO (%)	Repeat motif	No. of alleles/ individuals	Allele size range (bp)	H_O	H_E
Sper05	F: †AACAAAACTCACCCAACAT R: ATCCCTTCACGAAAATCACAG	64.0	4 mM	2	$(CA)_8TT(CA)_6$	9/46	169–184	0.6304	0.8175
Sper06	F: TCGCAGAGTGTGTTTGTGAGA R: ‡CGCACTCGCACTCGGATA	65.0	4 mM	2	$(AG)_2(TG)_2TT(TG)_2(AG)_2(TG)_4$ $(AG)_3AAGG(AG)_4$	4/46	194–197	0.3261	0.6082
Sper11	F: ‡AGCGTCACATTACTTACAAC R: TTTTCATCAGACTTACAATCA	56.0	4 mM	2	$(TCC)_2(TCT)_2(TCC)_5$	2/46	330–333	0.0217	0.0217
Sper14	F: †GGGACCCAATATAACC R: TAATCAAATCAATACATGCAC	56.0	2 mM	4	$(TGC)_8$ … $(TC)_7$	13/41	247–270	0.3902	0.8693*
Sper18	F: †AAATCTTTTTTCTGGACTCTT R: GCGTTTTTGGCATCTAT	50.6	4 mM	2	$(TG)_4AGC(AG)_2C(AGG)_3(A)_6$ $(AG)_3T(AG)_3TCT(AG)_4TTT(AG)_4$	3/46	293–297	0.2609	0.3084
Sper19	F: ‡CAATAATCTGAGCATCCGTTT R: CTTGGAGTGGGCTTACC	58.0	4 mM	4	$(GA)_4C(AG)_4C(GA)_3GG(GA)_5$	2/30	269–271	0.0333	0.0333

H_O, observed heterozygosity; H_E, expected heterozygosity; †, HEX labelled primer; ‡, 6-FAM labelled primer.
*Significant ($P < 0.05$) deviation from Hardy–Weinberg equilibrium.

fragments harbouring microsatellites were ligated into plasmids using the pGEM®-T Easy Vector kit (Promega) and transformed into JM109 competent cells. Insert-positive bacterial colonies were amplified using M13 primers and visualized by agarose gel electrophoresis. Approximately 120 amplicons containing inserts of 300–1000 bp were sequenced using an ABI PRISM 3730® DNA Analyser with a BigDye® version 3.1 kit (Applied Biosystems).

Twenty primer pairs were designed using OLIGO version 6.65, of which, six primer pairs reliably produced amplicons of an appropriate length (Table 1). A panel of 46 female specimens (preserved in 95% ethanol) from two collecting locations in California (Oxnard and Irvine) and six locations in Mexico (MX) and Guatemala (GT) (San Cristobal de las Casas, MX; Huayapam, MX; Puebla, MX; Coatapec Harinas, MX; Uruapan, MX; Chimaltenango, GT) was used to characterize these markers. Reactions were performed in a total volume of 25 μL, containing 2 μL DNA template (concentration not determined), 2.5 μL GeneAmp® 10× PCR Buffer II (Applied Biosystems), 20 μM each dNTP, 0.2 μM each primer, 1.2 μL Ampli*Taq* Gold® DNA Polymerase (Applied Biosystems), and varying concentrations of $MgCl_2$ and DMSO (Table 1). One primer from each pair was 5′ end-labelled with either HEX or 6-FAM (Sigma Genosys). PCR was performed in an Eppendorf Mastercycler® 5331 programmed for: 10 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature (Table 1) and 30 s at 72 °C. For two loci, Sper06 and Sper11, this was followed by a further 60 min at 72 °C to maximize nontemplated addition of a single adenosine nucleotide to the 3′ end of the amplicons (Smith *et al*. 1995). Amplicons were sized on an ABI PRISM 3100® Genetic Analyser, using GENEMAPPER® software version 3.0 and the internal-lane size standard GeneScan®-500 ROX™ (Applied Biosystems).

Heterozygosity estimates for all loci were calculated using POPGENE 1.32 (Yeh & Boyle 1997) (Table 1). Loci were tested for linkage disequilibrium (LD) and deviation from Hardy–Weinberg equilibrium (HWE) using a Markov chain method provided in GENEPOP 3.4 (Raymond & Rousset 1995). In the specimens sampled, two loci (Sper11 and Sper19) were essentially monomorphic, with a single (but different) specimen carrying a single copy of a second allele at each locus. In the remaining loci, the number of alleles per locus ranged from three to 13 and expected heterozygosity (H_E) from 0.31 to 0.87 (Table 1). Only one locus (Sper14) showed significant deviation from HWE, probably due to the presence of a null allele. However, given the relatively high polymorphism at this locus, the study sample may also have been too small to achieve a balanced HWE. No LD occurred between loci. Although essentially monomorphic, Sper19 may prove to be very informative for our origin study, with amplicons consistently produced in some populations but not others.

In addition to characterizing the six loci in *S. perseae*, the utility of the developed primers was tested in nine other pestiferous *Scirtothrips* species and two species from the closely related genus *Neohydatothrips* (Table 2). DNA samples of each species were tested under the PCR conditions optimized for amplification of the *S. perseae* samples. Presence of amplicons of an appropriate size was determined by agarose gel electrophoresis.

Table 2 Cross-species amplification using the six microsatellite primer pairs designed for *Scirtothrips perseae*. Where possible, DNA from two individuals of each species was tested

	Locus					
Species	Sper05	Sper06	Sper11	Sper14	Sper18	Sper19
S. aceri	—	—	*	—	—	—
S. astrictus	—	*	*	—	—	—
S. aurantii (South Africa)	—	—	±	±	±	—
S. aurantii (Australia)	—	—	—	—	—	—
S. citri	—	—	—	—	—	—
S. dorsalis (South Africa)†	—	—	—	—	—	—
S. dorsalis (India)†	—	—	—	—	—	—
S. sp. nr. *dobroskyi*	—	—	—	—	—	—
S. kenyensis	—	—	—	—	—	—
S. oligochaetus‡	—	—	—	—	—	—
Neohydatothrips burungae	—	—	±	—	—	—
N. geminus	—	—	—	—	—	—

*, successful amplification in both individuals; ±, successful amplification in only one individual; —, no amplification; †, specimen morphology matches *Scirtothrips dorsalis*, but molecular characterization of this 'species' is currently underway; ‡, only one specimen tested.

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