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PRIMER NOTE

Isolation and characterization of six polymorphic microsatellite loci in the western flower thrips *Frankliniella occidentalis* (Insecta, Thysanoptera)

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Abstract

Highly polymorphic microsatellite markers can provide important demographic information on founder events and range expansion following initial introduction of invasive insect species. Six microsatellite loci were isolated from an enriched DNA library in order to study the invasion patterns of the western flower thrips, *Frankliniella occidentalis*. All loci tested were found to be polymorphic and successfully amplified in all individuals. The number of alleles per locus ranged from five to nine and heterozygosity ranged from 45 to 73%. Some of the loci were also successfully amplified in other thrips species.

Keywords: *Frankliniella occidentalis*, genetic bottleneck, insect crop pest, invasive species, population genetic markers

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Thrips (Thysanoptera; Thripidae) are very small insects, widespread throughout the world with a preponderance of tropical species. Of the approximately 5000 species so far identified, a few hundred are crop pests, causing serious damage or transmitting diseases to growing crops and harvestable produce in most countries. Their minute size and cryptic behaviour make them difficult to detect either in the field or in fresh vegetation transported through international trade of vegetables, fruit and ornamental flowers. Consequently, many species have now spread from their original natural habitats and hosts to favourable new environments of valuable crops.

Frankliniella occidentalis (Pergande) was originally found in the USA in an area west of the Rocky Mountains between Mexico and Alaska (Bryan & Smith 1956). However, *F. occidentalis* has spread extremely rapidly since the mid-1980s and now occurs almost world-wide except in parts of tropical Africa and South America. Despite this interesting background and its commercial importance as a serious pest, genetic studies including *F. occidentalis* are scarce (e.g. Brunner *et al*. 2002; Forcioli *et al*. 2002). The very recent invasion history of *F. occidentalis* requires highly polymorphic markers to estimate genetic divergence between populations with different levels of pesticide resistance, to track the colonization routes of this pest and to identify sources of newly established populations. For this purpose, we developed polymorphic, codominant microsatellite markers for *F. occidentalis*.

Microsatellites were isolated using magnetic beads for hybridization enrichment. The protocols were based upon those provided by Hamilton *et al*. (1999) and Glenn *et al*. (2000) with the modification that we used commercially available kits wherever possible to increase repeatability. Briefly, genomic DNA was extracted from approximately 100 pooled *F. occidentalis* from a laboratory strain using the GenElute DNA extraction kit (Sigma) following the manufacturer's specifications. The extracted DNA was digested with *Rsa*I (New England BioLabs), and ligated to the 'linker-oligonucleotides' SNX-F and SNX-R (5′-CTAAGGCCTTGCTAGCAGAAGC-3′; 5′-pGCTTCTGC-TAGCAAGGCCTTAGAAAA-3′) using a DNA ligation kit (Fermentas). Fragments 300–700 bp in length were recovered following size fractionation by agarose-gel electrophoresis and extraction with the QIAquick gel extraction kit (Qiagen). Following a linker-primed polymerase chain reaction (PCR), insert DNA was denatured and hybridized to simple-sequence repeat oligonucleotides [$(CA)_{10}$, $(CT)_{10}$ and $(AAG)_8$] that were 5′-biotinylated. Streptavidin-coated beads (Pierce, MagnaBind) were added and incubated at room temperature to capture DNA fragments with microsatellite sequences complementary to the microsatellite oligos

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Table 1 Characteristics of six microsatellite loci from *Frankliniella occidentalis*. Allele number and heterozygosities (H_O, observed; H_E, expected) were calculated on 25 individuals from Stanthorpe, Australia. Fluorescent-labelled primers are indicated by either 'h-' (HEX) or 'f-' (6-FAM, Sigma). T_a, annealing temperature. GenBank Accession nos: AY629237–AY629242

Locus name	Repeat motif	Primer sequence (5′–3′)	H_O	H_E	Fragment size range	No. of alleles	T_a (°C)
FOCC44	$(GT)_{49}$	TGTCACCAAGGCGGTGG	0.45	0.73	90–105	5	60
		h-CGCTGGACCTTACCGAGAGA					
FOCC55	$(CTT)_{16}$	GGCATGTCTCAGCTTCGTCA	0.55	0.75	228–273	6	60
		h-AGGAAGGGTATAGGAAATGCAGG					
FOCC56	$(GA)_{42}$	TCAACCCCCATCACTCTTCC	0.68	0.58	193–209	7	60
		f-CCTTGAGCTCCCCTCACCTC					
FOCC75	$(GA)_{23}$	GGATATTATTTTCCCGTCCCG	0.73	0.84	184–244	9	60
		f-TGGTTCTTTTGTAAAGGCAGCG					
FOCC83	$(GT)_{46}$	GTCTGTCACCAAGGCGGTGG	0.68	0.80	72–90	7	60
		f-CAGGTAACGCACAGTGCTGCTC					
FOCC125	$(GC)_6(GT)_{10}$	AACCCGCACCGTGCA	0.68	0.75	138–156	6	60
		f-AGTTGGGCTGCCGTCC					

(probes). Beads and attached probes were separated magnetically from the supernatant. Following stringent washes, the bound DNA was recovered by incubating in Tris-Low-EDTA at 95 °C for 5 min.

The recovered DNA was prepared for TA cloning (Qiagen, PCR cloning kit) by another linker-primed PCR. Positive (ampicillin selection) clones were picked, transferred to tubes containing 100 μL ddH_2O, and heated for 10 min at 90 °C to release the plasmid DNA. Subsequent PCR amplifications were carried out using the standard Primers M13 (–26) reverse and M13 (–21) forward. The resulting PCR fragments were analysed on an ABI PRISM® 3100 (Applied Biosystems) autosequencer by cycle sequencing using BigDye Terminator (Applied Biosystems). A total of 130 clones were sequenced and specific primers and PCR conditions were defined for six loci. Technical details and GenBank Accession nos are given in Table 1. The amplifications were performed on 25 individuals from one population (Stanthorpe, Australia). Amplifications were carried out in a 20 μL reaction mix containing 25–50 ng template DNA, 0.5 μM each primer and the HotStarTaq™ Master Mix (Qiagen). The latter contained 2 U of HotStarTaq™ DNA polymerase, 200 μM each dNTP, and PCR buffer with a final concentration of 1.5 mM MgCl. Either the forward or the reverse of each primer set was 5′ end-labelled with either 6-FAM or HEX (Sigma) for laser-beam detection of the PCR products. PCR amplification was performed using the TC-412 (Techne) thermal cycler with the following protocol: 15 min at 95 °C, followed by 35 cycles of 45 s at 95 °C, 1 min annealing temperature at 60 °C and 30 s at 72 °C.

PCR products were sized on an ABI PRISM® 3100 Genetic Analyser using GENESCAN software (Applied Biosystems) and the internal-lane size standard GeneScan –500 ROX. Heterozygosity estimates for all loci were determined using ARLEQUIN 2.0 (Schneider *et al*. 2000). Exact tests of linkage disequilibrium and deviation from Hardy–Weinberg equilibrium (HWE) were calculated using a Markov-chain method provided in GENEPOP 3.4 (Raymond & Rousset 1995).

All loci tested were found to be polymorphic and successfully amplified in all individuals. The number of alleles per locus ranged from five to nine and heterozygosity ranged from 45 to 73% (Table 1). Following Bonferroni correction, significant linkage disequilibrium was only observed between loci FOCC44 and FOCC83. At three of the loci (FOCC44, FOCC55, FOCC83), observed (H_O) and expected heterozygosity (H_E) values did not conform to HWE expectations due to heterozygote deficits (Table 1). Nevertheless, these values are encouraging for upcoming population genetic studies because *F. occidentalis* was introduced to Australia as recently as in 1993. Thus, the Stanthorpe population very likely shows reduced variability compared to natural populations due to this recent genetic bottleneck.

DNA from 10 other thrips species was prepared according to the above procedure and used to test transferability of the six microsatellite primers (Table 2). Amplification conditions were identical to those used in *F. occidentalis* with the exception that annealing temperature was lowered to 50 °C. PCR products were generated in many of the assayed species. One locus (FOCC83) was polymorphic in all species.

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Table 2 Cross-species amplification at the six microsatellite loci. Number of alleles detected at each locus using samples of five individuals from each of 10 thrips species. A dash indicates failure of amplification

	Locus					
Species	FOCC44	FOCC55	FOCC56	FOCC75	FOCC83	FOCC125
Echinothrips americanus	—	—	—	1	3	2
Frankliniella tritici	4	1	1	1	3	3
Frankliniella bispinosa	4	—	1	3	4	1
Franklinothrips vespiformis	1	1	3	—	3	3
Hercinothrips femoralis	3	2	2	1	3	1
Parthenothrips dracaenae	1	2	—	2	2	1
Thrips flavus	1	—	1	1	2	1
Thrips fuscipennis	—	2	—	—	3	3
Thrips palmi	1	—	—	2	2	1
Thrips tabaci	2	1	1	4	4	3

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