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Final Report

Molecular ecology of pest thrips

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CSIRO Entomology

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TECHNICAL SUMMARY

Six polymorphic microsatellite primer pairs have been developed for *Frankliniella schultzei* and 5 polymorphic microsatellite loci for *Frankliniella occidentalis*. This has provided a total of 11 loci for use in *Frankliniella.* These eleven loci have been screened across the 30 individual insects supplied. These primers clearly differentiate the 6 screened populations. The preliminary genetic data indicates that population FoE is probably a mixed population containing individuals of both *F. schultzei* and *F. occidentalis.* This would need to be verified by morphological assessment. These microsatellites are a useful diagnostic and population genetics tool for both *Frankliniella* species.

The microsatellite primers have been designed on genomic sequences belonging to two *Frankliniella* species, and as such some of the microsatellites work most efficiently in the species from which they were derived. Although the microsatellites transferred across species in some cases, the products were of a different size and did not amplify as reliably as in the species from which it was sourced. This indicates reasonable genetic distance between the species tested.

MEDIA SUMMARY

The project aimed to construct two microsatellite libraries for two related species of thrips, *Frankliniella schultzei* and *Frankliniella occidentalis.* A total of six functional and polymorphic microsatellite primer pairs for *Frankliniella schultzei* and five 5 functional and polymorphic microsatellite primer pairs for *Frankliniella occidentalis* were produced. The microsatellite primers have been designed on genomic sequences belonging to the two *Frankliniella* species, and as such some of the microsatellites work most efficiently in the species from which they were derived. Although the microsatellites transferred across species in some cases, the products were of a different size and did not amplify as reliably as in the species from which it was sourced. This indicates reasonable genetic distance between the species tested. The results suggested possible h on the surface conflicts with #2 below and suggests that possible hybridisation between the two species had occurred some time ago.

INTRODUCTION

The western flower thrips, *Frankliniella occidentalis*, is native to North America, but has now spread to many countries throughout the world. It has been found in most European countries, Japan, Kenya, South Africa, Hawaii, Costa Rica, Colombia, New Zealand, and now Australia. This species of thrips is primarily a pest in greenhouses, but is capable of causing severe damage to a variety of field crops. As well as attacking produce directly, it is a major vector of several viral diseases of plants. It has many hosts, having being recorded from 244 plant species.

The yellow flower thrip, *Frankliniella schultzei* occurs in pale and dark forms. The pale form female is yellow with brownish blotches. The dark form is uniformly dark brown. *F. schultzei* occurs in tropical and subtropical areas. The dark form occurs worldwide and is also known from Florida.

This project aimed to develop two microsatellite enriched libraries, one in *Frankliniella occidentalis,* and one in *Frankliniella schultzei.* Five microsatellite loci are to be developed for each species and screened across a panel of 30 individuals derived from 6 populations.

MATERIALS AND METHODS

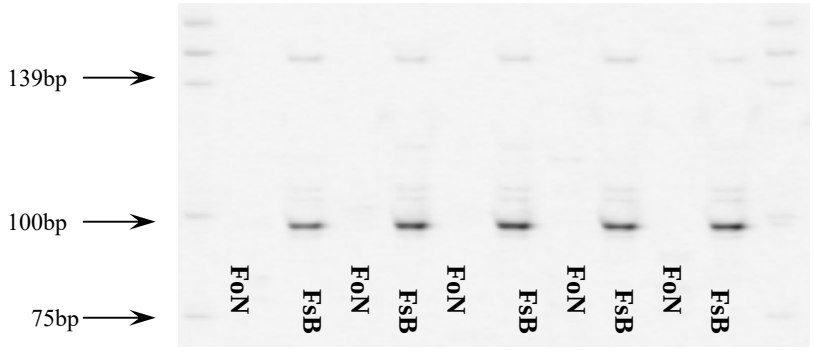
Details of the material used in screening are shown in Appendix 1.

The construction of two microsatellite libraries (*Frankliniella schultzei* and *Frankliniella occidentalis*), used Qiagen Tissue Extraction Kit for the DNA extractions from bulks of individuals. The microsatellite library construction involved restriction of the bulk genomic DNA, the ligation of adaptors to the genomic DNA and the amplification of the DNA by PCR. The amplification products were then hybridized to biotin-labeled microsatellite oligonucleotides attached to magnetic beads. The magnetic bead captured and enriched genomic products were then amplified again using PCR, and cloned. Library clones were sequenced to determine which contained a microsatellite within the insert. Clones, which contained microsatellites, were sequenced in the reverse orientation. Resulting sequence information was then used for microsatellite primer design. Details of the microsatellites derived by this method and used in the final screening are shown in Appendix 2. The microsatellite primers were initially tested on a selection of 8 *Frankliniella* individuals representing both species. The primers that amplified products of the expected size were then used for the microsatellite screening of 5 individuals from each of the 6 populations defined in Appendix 1 (30 individuals in total).

For the microsatellite analysis the DNA was extracted from individuals using the Chelex protocol (Walsh *et al.* 1991). Amplifications conditions were as follows: 1µl Chelex DNA, 4mM $MgCl_2$, 0.2µM of each primer, 20mM Tris-HCl, 100mM KCl, 1 unit of *Taq* (Qiagen) and 0.2mM of dNTPs in a 20µl reaction volume. Cycling conditions in a Corbett Research Thermal Cycler were 94°C for 40sec, 50°C /55°C for 40sec, 73°C for 40sec for 35 cycles (see Appendix 2 for Ta temperature for each primer pair). Amplification was verified on agarose.

Microsatellite scoring was on 7% native acrylamide in a GS2000 Genetic Analyser (Corbett Research). An example of a GS2000 result is shown below for primer shu2 (Fig.1). The absolute sizes derived from the GS2000 were converted to allele sizes (ie. multiples of the SSR repeat length from the expected size), see Appendix 4 for all scored alleles. As an example of allele size scoring: in a dinucleotide SSR, such as for schu7 which had an expected size of 112bp: allele sizes are given as 114bp, 116bp, 118bp etc. The absolute scores derived from the GS2000 may thus be rounded up by 1 base pair if necessary. In the example of a trinucleotide SSR such as for schu2 which has an expected size of 100bp alleles, then allele scores will be 103bp, 106bp, 109bp etc., with the absolute scores being rounded up or down 1bp as necessary.

Figure 1. Amplification products of the microsatellite locus schu2 from collections *Frankliniella occidentalis* (Narrabri - FoN) and *Frankliniella schultzei* (Bowen - FsB). Five individuals from each collection are shown.



RESULTS

The 11 microsatellite primers developed in this research project have been screened over the six populations (30 individuals) defined in Appendix 1, and have shown to be polymorphic both within and between populations (see Appendix 2 for primer details). The utility of the microsatellites across the populations did differ. The microsatellite primers worked most efficiently in populations which were of the same species as which the microsatellite locus was derived (see Appendix 3 for microsatellite origin).

In populations of *Frankliniella* where the species was not the same as the species from which the microsatellite primer was derived, the amplifications were still generally good. The PCR product sizes however, were often further from the expected size and the amplifications were not as frequent. This is often the case for microsatellites when they are transferred across to related groups, and indicates a significant genetic distance between the *Frankliniella* species. Where the PCR product is very different from the expected size, sequencing should be used to verify that it is the equivalent locus in the related group in which it is being applied.

The preliminary genetic data presented here indicates that population FoE (*Frankliniella occidentalis* – from Emerald) is most likely a mixed population containing individuals of both *F. schultzei* (FoE1, FoE3 and FoE5), *F. occidentalis* (FoE2) and perhaps a third unknown species (FoE4). Population FsB (*Frankliniella schultzei* – Bowen) also has some unusual amplification patterns and would seem to have characters in common with both *F. schultzei* and *F. occidentalis.*

SUMMARY OF SCREENING

1) *Frankliniella occidentalis* and *F. schultzei* can be separated by alleles at several loci as there is no overlap between the allele ranges. In particular, Fo11 and Fo15 (assuming Bowen is actually *F. occidentalis*).
2) Bowen population of *F. schultzei* is odd in that it has within an individual locus, alleles characteristic of *F. schultzei* and loci where the alleles are characteristic of *F. occidentalis*. This suggests that the species separation between the two may not be clear cut.
3) Narrabri *F. occientalis* looks more like *F. schultzei* while Bowen looks more like *F. occidentalis*. It is possible that the samples received had been mislabeled and this is being checked.
4) Emerald has a mixed population.
5) The sample size needs to be increased in order to clarify matters and we will need to recheck the Bowen, Emerald and Narrabri samples to confirm their IDs.

Table 1. Allele size range for each of the 11 loci.

	Fo2	Fo5	Fo10	Fo11	Fo15	Fs2	Fs3	Fs5	Fs7	Fs10	Fs12
F. occidentalis											
Brookstead	144-154	103-107	114-116	110-116	151-163	172-220	0	232-278	106-144	0	112
Emerald	144-152	103-107	114	112-116	149	175	0	236	0	0	112
Narrabri	154	163	132-212	0	0	85-208	160-162	182-280	108-138	107-115	106-109
WA	142-152	103	114-116	114-138	151-233	118-178	136-160	156-278	114	107-147	112-115
F. schultzei											
Emerald	152-154	99-163	130-214	0	0	97	118-120	192-194	108-116	107-115	106-112
Bowen	142-154	103-107	114-116	110-124	165-215	97	118-120	192	108-116	0	112
Kununurra	154-162	103-163	132-212	0	0	82-148	118-138	192-246	108-116	105-121	106-112

DISCUSSION

Six polymorphic microsatellite primer pairs have been developed for *Frankliniella schultzei* and 5 polymorphic microsatellite loci for *Frankliniella occidentalis*. This has provided a total of 11 loci for use in *Frankliniella.* These eleven loci have been screened across the 30 individual insects supplied. These primers clearly differentiate the 6 screened populations. The preliminary genetic data indicates that population FoE is probably a mixed population containing individuals of both *F. schultzei* and *F. occidentalis.* This would need to be verified by morphological assessment. These microsatellites are a useful diagnostic and population genetics tool for both *Frankliniella* species.

PROJECT LIMITATIONS

The microsatellite primers have been designed on genomic sequences belonging to two *Frankliniella* species, and as such some of the microsatellites work most efficiently in the species from which they were derived. Although the microsatellites transferred across species in some cases, the products were of a different size and did not amplify as reliably as in the species from which it was sourced. This indicates reasonable genetic distance between the species tested.

REFERENCES

Doyle,J.J., Doyle,J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* **12:**13-15.

Walsh,P.S., Metzger,D.A., Higuchi,R. (1991) Chelex®100 as a medium for simple extraction of DNA for PCR based typing from forensic material. *Biotechniques* **10**: 506-513.

TECHNOLOGY TRANSFER

The project in this first stage sought only to develop the microsatellites. Future research will be needed in order to use the microsatellites to address issues that will deliver benefits to growers.

RECOMMENDATIONS

Western flower thrips is a pest that has a wide range of crops and non-crops, most of which are short lived. Therefore, WFT needs to move between hosts if it is to survive. This has a number of implications in terms of improving pest management, these include,

1) Knowing what are the major sources of infestation by the thrip and determining whether these are also sources of infection by TSWV will provide growers with the knowledge of how to adapt their farm management to minimise thrips outbreaks at key times in their production cycle.

2) Knowing how thrips move across a wide geographic area e.g. the Lockyer Valley may contribute to developing more effective insecticide resistance management programs.

3) As the cotton industry has shown, understanding how the landscape contributes to the development of effective areawide management and insecticide resistance management has been central to their capacity to continue to produce profitably.

It is suggested that the work be continued as a joint PhD project with CSIRO and the Spatial Ecology group at UQ. There are opportunities to link this project to two others, one a GRDC funded project on silverleaf whitefly and the other of a CRDC

funded project on green mired; both seeks to address similar questions using microsatellites. The PhD would seek to
1) resolve the uncertainties over the microsatellites as well as utilise another set of microsatellites developed by a lab overseas
2) investigate the role that different farming systems play in the genetic structure of WFT populations. Here one could use a protected cropping system e.g. one in SA, an intensive cropping system in SE Qld and another in Central Qld.

APPENDIX 1

SAMPLE LIST

The collections used for microsatellite library construction are shown with an asterisk. Individuals within each of the screened collections were labeled 1 to 5.

Species	Location	Population Code
*Frankliniella occidentalis**	(WFT)	
*Frankliniella schultzei**	Emerald (pale form)	
Frankliniella occidentalis	Brookstead	FoBk (1-5)
Frankliniella occidentalis	Emerald	FoE (1-5)
Frankliniella occidentalis	Narrabri	FoN (1-5)
Frankliniella occidentalis	Western Australia	FoWA (1-5)
Frankliniella schultzei	Bowen	FsB (1-5)
Frankliniella schultzei	Kununurra (pale form)	FsK (1-5)

APPENDIX 2

MICROSATELLITE PRIMER DETAILS

Microsatellites where the interruption of the repeat is greater than 2 base pairs is shown by a colon.

Primer	Microsatellite	Primer Forward	Ta (ºC)	Primer Reverse	Ta (ºC)	Exp. size	PCR - Ta (ºC)
occid2	(CT)5TT(CT)5G(TC)4	CGGCAGACAAGCACCACA	53	AATGAGGCTGGACGTATGAG	55	154bp	Ta=50
occid5	(TCCC)5	CAACCCAGCGCTTACTCGCTC	63	GCAGAGTGATCAGTGATCAGC	59	111bp	Ta=55
occid10	(AC)4:(AC)7:(AC)3	AAAGCCAGTGCAGGCATG	55	ACGTATGCTCGCATGTAT	47	120bp	Ta=50
occid11	(CA)13	CACACAGGGAAGGCCGAG	55	AATCGGCTTTGTATGCATTCA	51	122bp	Ta=55
occid15	(TC)6TG(TC)4	CCTAACAGCTGCATTGCAC	53	CACTGAGTCTATAAGCACCG	55	169bp	Ta=50
schu2	(CAC)8	ACGTCATAACGCGCGATCC	51	CTGACCTCTAGCAGTTGC	53	100bp	Ta=50
schu3	(CA)8CC(CT)5	AGAGCGCAGCACTGCAACA	55	ACCTCGATAAATCTCAGGAAG	55	124bp	Ta=50
schu5	(CA)6AA(CA)4	CAGTCGCTGCCGTAGTCG	55	TGCGCGAGGCACGAGTAG	55	196bp	Ta=50
schu7	(GT)10	GAGCACCTGACATCGAGC	53	CGCACCGAAATAGGCATC	51	112bp	Ta=50
schu10	(CT)3(CA)7	CCGCCATGCCAGATATATGT	55	GTGGTCCAGTCCGTGGAA	53	111bp	Ta=50
schu12	(GTG)5	GTCCACGACGAATCTAGGC	55	TCGTTTGAATCAGTGTGACTC	55	115bp	Ta=50

APPENDIX 3

MICROSATELLITE DEVELOPMENT DETAILS

Microsatellites where the interruption of the repeat is greater than 2 base pairs is shown by a colon

Primer	Microsatellite	Source species	Number of Alleles
occid2	(CT)5TT(CT)5G(TC)4	*Frankliniella occidentalis*	7
occid5	(TCCC)5	*Frankliniella occidentalis*	6
occid10	(AC)4:(AC)7:(AC)3	*Frankliniella occidentalis*	7
occid11	(CA)13	*Frankliniella occidentalis*	13
occid15	(TC)6TG(TC)4	*Frankliniella occidentalis*	12
schu2	(CAC)8	*Frankliniella schultzei*	12
schu3	(CA)8CC(CT)5	*Frankliniella schultzei*	6
schu5	(CA)6AA(CA)4	*Frankliniella schultzei*	16
schu7	(GT)10	*Frankliniella schultzei*	12
schu10	(CT)3(CA)7	*Frankliniella schultzei*	10
schu12	(GTG)5	*Frankliniella schultzei*	4

MICROSATELLITE ALLELE SIZES

Microsatellite primers are in columns, and individuals in rows. No amplification is shown by a (0). Individuals are described by population code (see Appendix 1) followed by individuals numbered 1-5 for each population.

	occid2	occid5	occid10	occid11	occid15	schu2	schu3	schu5	schu7	schu10	schu12
FoBk1	148/152	103/107	114	110	163	172	0	0	0	0	112
FoBk2	148	103	114	112	161	175	0	232/278	106/122	0	112
FoBk3	150/154	103/107	114	116	0	175	0	234	110/144	151	112
FoBk4	152	103	114	112/116	151	175/220	0	0	120	0	112
FoBk5	144/150	103	116	112/116	165	175	0	0	124	0	112
FoE1	152	159	130/110	0	0	97	118	192	108/114	107/113	106
FoE2	144/152	103/107	114	112/116	149	175	0	236	0	0	112
FoE3	154	99/163	132/214	0	0	97	118	194	108/114	111/115	106/109
FoE4	0	0	0	0	0	0	0	0	0	0	91
FoE5	154	99/163	214	0	0	97	120	194	108/116	107/115	106/112
FoN1	154	163	132/212	0	0	85	160	182/210	108/124	107/115	106/109
FoN2	154	163	132/212	0	0	175/208	162	208/260	138	109/113	106/109
FoN3	154	163	132/214	0	0	0	0	0	0	109/115	106/109
FoN4	154	163	132/212	0	0	115/175	0	232/280	124/128	109/113	106/109
FoN5	154	163	132/214	0	0	175	0	0	0	109/113	106/109

	occid2	occid5	occid10	occid11	occid15	schu2	schu3	schu5	schu7	schu10	schu12
FsB1	142	103	116	110	165	97	118	192	108/116	0	112
FsB2	150/152	103	114	112/114	187	97	120	192	108/116	0	112
FsB3	148	103	0	0	0	97	120	192	108/116	0	0
FsB4	150/154	103/107	116	118/124	167	97	120	192	108/116	0	112
FsB5	152	103	114	112	215	97	120	192	108/114	0	112
FsK1	154/162	163	132/212	144	0	97	118	192	108/116	107/113	106/112
FsK2	154	163	132/212	0	209	82/148	118/136	192/246	108/112	107/123	109
FsK3	154	163	132/212	0	0	97	118	192	108/116	107/115	109/112
FsK4	154	167	132/212	0	0	82/97	118/138	192/220	108/116	105/121	109/112
FsK5	154	103/163	132/212	0	0	97	118	192	108/114	107/113	106/109
FoWA1	142	103	116	128/138	167	178	160	0	114	0	112
FoWA2	152	103	116	122/132	153/233	118/178	0	158/232	0	0	115
FoWA3	152	103	114	116/126	151/213	175	136	234/278	0	107/113	112
FoWA4	142	103	114	114/122	167	175	0	156/260	0	0	112
FoWA5	152	103	114	116/130	165	0	0	230/278	0	147	112