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PRIMER NOTES

Microsatellite loci for the social wasp *Polistes dominulus* and their application in other polistine wasps

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The social wasps of the genus *Polistes* are an important model system for understanding the evolution of cooperation. Their relatively simple societies lack the distinct morphological castes which characterize many of the social insects, and newly emerged females possess a variety of reproductive options (Reeve 1991). A female may remain on her natal nest as a helper gaining indirect fitness; usurp a foreign nest and become reproductively dominant; initiate a new nest independently; reproduce on a satellite nest; or initiate a new nest in cooperation with other wasps (Strassmann 1981; Reeve 1991; Mead *et al*. 1995; Cervo & Lorenzi 1996; Queller *et al*. 2000). By characterizing the reproductive payoffs associated with different reproductive strategies, we are better able to understand how cooperative societies are maintained.

Recently, microsatellite genetic loci have greatly extended our ability to characterize the reproductive strategies used by social wasps (Hughes 1998; Queller *et al*. 1993a). Using microsatellite loci we can reconstruct pedigrees, and estimate relatedness. Using this information, unobserved events such as queen death, nest usurpation or past reproductive dominance can be inferred (Queller *et al*. 1993a,b; Field *et al*. 1998; Hughes 1998). In this paper, I describe microsatellite loci isolated from the social wasp *Polistes dominulus*, one of the best studied *Polistes* species.

We followed published protocols for the isolation of microsatellite loci (Strassmann *et al*. 1996) with clarifications and modifications to those protocols as noted below. DNA was extracted from 1 to 1.5 g of pupal thoraces ground in a mortar and pestle which had been chilled in liquid nitrogen. The ground tissue was suspended in grinding buffer (0.1 M NaCl; 0.1 M Tris-HCl, pH 9.1; 0.05 M EDTA; 0.05% SDS), and purified three times with phenol:chloroform:isoamyl alcohol (25:24:1), and then three times with chloroform:isoamyl alcohol (24:1). The purified genomic DNA was then ethanol precipitated, and resuspended in distilled water.

Genomic DNA was digested with *Sau*3aI, and 300–1000 bp inserts were ligated into the pZErO –2 plasmid (Zero Background cloning kit, Invitrogen) digested with *Bam*HI. We transformed TOP10 cells (Invitrogen) to obtain approximately 5000–6000 clones. Nylon replicates of the genomic library were probed with five oligonucleotides (AAT_{10}, AAG_{10}, AAC_{10}, TAG_{10}, and CAT_{10}) which were end-labelled with [γ-^{33}P]-dATP. Probes of the nylon replicates yielded 151 positives and subsequent probing of plasmid DNA on the southern blot confirmed 34 unique positives. Clones which were positive on the southern blot were sequenced on an ABI 377 automated sequencer (Perkin-Elmer), and 19 sets of polymerase chain reaction (PCR) primers were designed from the 28 resulting sequences using Mac Ventor 5.0 (Kodak Scientific Imaging Systems).

We optimized the PCR primers on an MJ Research PTC-100 thermocycler using 10 µL reactions (Peters *et al*. 1998), and assessed within-species polymorphisms for eight species of polistine wasps, using from one to eight unrelated females for each species (Table 1). PCR products were visualized on 6% polyacrylamide/8 M Urea sequencing gels.

Twelve of the 19 loci tested were polymorphic within our *P. dominulus* population and had a mean observed heterozygosity (H_O) of 0.76. Loci with a minimum of five uninterrupted repeats were polymorphic, and heterozygosity increased logarithmically with the number of uninterrupted repeats (Fig. 1; logarithmic regression, $R^2 = 0.454$, $P = 0.0016$). The loci retained much of their polymorphism in other species of *Polistes* with six polymorphic loci for *P. fuscatus* and *P. apachus* which had a mean H_O of 0.48. No polymorphisms were detected outside of the *Polistes* genus, however, it is likely that some polymorphisms went undetected due to the small number of individuals screened in the other species (Table 1).

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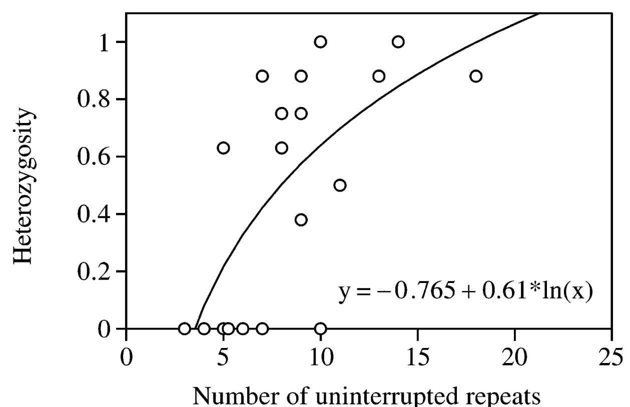

Fig. 1 The relationship between the observed heterozygosity and the number of uninterrupted repeats for 19 microsatellite loci isolated from *Polistes dominulus*.

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Table 1 A description of polymorphic microsatellite loci isolated from *Polistes dominulus*, including their utility in related polistine taxa. The sample size (n) for each species is given in the column heading with exceptions for certain primers noted in the table. Where $n \geq 3$, we report the observed heterozygosity for all species, as well as the expected heterozygosity for *P. dominulus*. In all cases we report the observed number of alleles in parentheses. The product size and repeat region data are based on the sequenced allele. NP = no scorable product. GenBank accession nos are AF155596 to AF155623 and include 16 additional loci not summarized in the table

Locus	Size (bp)	T_a (°C)	Repeat	*Polistes dominulus* (n = 8)	*Polistes fuscatus* (n = 4)	*Polistes apachus* (n = 4)	*Protopolybia exigua* (n = 2)	*Brachgastera mellifica* (n = 2)	*Polybia occidentallis* (n = 1)	*Ropalidia excavata* (n = 1)	*Miscocyterus alfkenii* (n = 1)	Primers (5′–3′)
Pdom 1	209	55	$(CAG)_9TAG(CAG)_5$ $(CAT)_5GGCAC(CAG)_3$	H_O = 0.38 (3) H_E = 0.41	0.00 (1)	–(2) n = 1	–(1)	–(1)	–(1)	–(1)	–(1)	F:GGACGCTCGGCTGATTTGTC R:AAGGGATTTTTCCTGAGACTATTCG
Pdom 2	184	51, 48	$(AAG)_8CG(AAG)_2$	H_O = 0.75 (4) H_E = 0.63	0.50 (4)	0.33 (3)	NP	NP	NP	NP	NP	F:CGTCTCTCGAAATATGCTAAAC R:AGAACGGTAAACATTCTTCTATC
Pdom 7	160	54	$(AAG)CAG(AAG)_9$	H_O = 0.75 (5) H_E = 0.73	–(1) n = 2	0.00 (1)	–(1) n = 1	–(1) n = 1	–(1)	NP	–(1)	F:CACTGTATTGTCCTACGGTGGTCC R:GCGAGAACCTGTACTCAAAACAAAC
Pdom 20	236	55, 52	$(CAT)_{18}$	H_O = 0.88 (4) H_E = 0.63	0.75 (6)	1.00 (5)	NP	NP	NP	NP	NP	F:TTCTCTGGCGAGCTGCACTC R:AGATGGCATCGTTTGAAAGAGC
Pdom 25	157	50, 45	$(AAG)_{11}$	H_O = 0.50 (3) H_E = 0.53	NP	NP	NP	NP	NP	NP	NP	F:CATTATAAACGCCGCG R:ACGATGGAAACGTAAGTCC
Pdom 93	131	55	$(AAG)_2ACG(AAG)_2$ $ACG(AAG)_5$	H_O = 0.63 (2) H_E = 0.43	0.25 (4)	0.50 (5)	–(1)	–(1)	NP	–(1)	NP	F:CCATCAGCTGTCCCATTCGC R:AATCGGTTTCGCTCGTCCACCTCC
Pdom 117	260	51, 48	$(AAG)_4AGG(AAG)_2$ $AGG(AAG)_{14}$	H_O = 1.00 (9) H_E = 0.83	0.25 (2)	–(2) n = 2	–(1)	–(1)	NP	NP	NP	F:AAGAAAACCTACTACGTTGTGTGAG R:TTTCAACATTCCATAGGGACAG
Pdom 121	218	54, 50	$(AAG)_8AGGAAC$ $(AAG)_2AAC(AAG)_2$	H_O = 0.63 (6) H_E = 0.78	0.00 (1)	0.00 (1)	NP	NP	NP	NP	–(1)	F:GAGTGGGTATGACGAAGATGATGG R:TGATTATAGCCTGCCGAAACTCTG
Pdom 122	172	46, 48	$(AAT)_{10}GAAAAT$ $(AAT)_2GAAAAT\ (AAT)_8$	H_O = 1.00 (9) H_E = 0.85	0.50 (2)	–(2) n = 1	NP	NP	NP	NP	–(1)	F:CCGAAGAATGATAGTAGGTCC R:AGACCATCTCTCGCACGC
Pdom 127b	119	48	$(AAT)_{13}\ldots(AAT)_6\ AA$ $(AAT)_4AAC(AAT)$	H_O = 0.88 (9) H_E = 0.80	0.00 (1)	0.00 (1)	–(1)	–(1)	–(1)	–(1)	–(1)	F:TCCCCCGTTTTTGGTCCTTG R:GGGAGAGAATCGTGCCTTTTC
Pdom 139	186	48, 45	$(AAC)_7(AAT)_2(AAC)$ $(AAT)_2(AAC)_2$	H_O = 0.88 (6) H_E = 0.72	0.00 (1)	0.00 (1)	NP	NP	NP	NP	– (1)	F:TGACAAAAGACAACAAAATATG R:AGCTTCGGTAGGGCTTCG
Pdom 140	192	55	$(TAG)_9$	H_O = 0.88 (9) H_E = 0.85	0.00 (1)	0.00 (1)	–(1)	–(1)	–(1)	NP	NP	F:GCTTTTCCCTTATTTTCCCG R:CGTGTTCGTATATTCCTGTAACG
Pdom 151	115	52, 50	$(CAT)_2AA(CAT)CAAT$ $(CAT)_3$	H_O = 0.00 (1) H_E = 0.00	0.25 (2)	–(1)	–(1)	–(1)	NP	–(2)	–(1)	F:TGATGTTACCACTGCTTTGAGCG R:TTCAGCACCGTCGTCGTTGTTG

T_a, annealing temperature.

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Characterization of nuclear microsatellites in *Pinus halepensis* Mill. and their inheritance in *P. halepensis* and *Pinus brutia* Ten.

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Nuclear microsatellites, or single sequence repeats (nSSRs), have been characterized in many tree species and are powerful markers for genetic diversity studies in natural populations (e.g. Echt *et al.* 1996; Pfeiffer *et al.* 1997). Although nSSR enrichment protocols have successfully been applied to conifers (Edwards *et al.* 1996), identification of single-locus, reproducible markers is difficult, probably because of their large genome size and complexity (Pfeiffer *et al.* 1997; Soranzo *et al.* 1998). In this study, we report the successful isolation of nSSRs in *Pinus halepensis* Mill. and their Mendelian segregation in both *P. halepensis* and *P. brutia*, two closely related Mediterranean pines.

A microsatellite library enriched for di- (GC, CT, CA), tri- (CAA, GCC) and tetra-nucleotide (GATA, CATA) repeats was constructed for *Pinus halepensis*, following the method described by Edwards *et al.* (1996). A total of 43 clones containing a microsatellite were detected from 47 clones randomly chosen from the library: 16% were repetitions of a single nucleotide (A/T), 77% were repetitions of dinucleotides (CA, CT or compounds CA–TA, CA–GA) and 7% were repetitions of trinucleotides (TAA, GCC). Sequencing reactions were performed using the Pharmacia AutoRead Sequencing Kit, and run on a 6% polyacrylamide gel containing 7 M urea using an ALF Pharmacia automatic sequencer. Primers were designed for the amplification of 25 dinucleotide nSSRs using the computer program Primer (http://www-genome.wi.mit.edu/genome_software/other/primer3.html).

Total genomic DNA extracted from leaf and mega-gametophyte tissue was used for testing the primer pairs. The procedure described by Doyle and Doyle (1990) and the Nucleon Phytopur DNA extraction kit were used for leaf tissue and mega-gametophytes, respectively. Polymerase chain reaction (PCR) was carried out using a Gradient 96 Stratagene Robocycler: the reaction solution (25 μL) contained four dNTPs (each 0.2 mM), 0.25 μM of each primer, 2.5 μL reaction buffer (100 mM Tris–HCl pH 9.0, 15 mM $MgCl_2$, 500 mM KCl), 25 ng of template DNA and 1 unit of Taq polymerase (Pharmacia). After a preliminary denaturing step at 95 °C for 1.5 min, PCR amplification was performed for 35 cycles: 1.5 min denaturing at 94 °C, 1.5 min at annealing temperature (Table 1) and 1.5 min extension at 72 °C, with a final 5 min step at 72 °C. After amplification, PCR products were mixed with a loading buffer (98% formamide, 10 mM EDTA pH 8.0, 0.1% bromophenol blue, 0.1% xylene cyanol and 10 mM NaOH), heated for 5 min at 95 °C, and then set on ice. Fragments were electrophoretically separated on a 6% polyacrylamide gel and stained using silver nitrate (Rajora *et al.* 2000).

Out of 25 primer pairs, nine (36%) either gave no amplification ($n = 4$) or produced multi-band patterns ($n = 5$). Sixteen produced fragment amplification in the expected size range, of which eight were polymorphic within one or the other species (Table 1). This proportion of functional markers is comparable to what is generally observed in conifers (e.g. Echt