Polymorphic microsatellite loci for primitively eusocial wasp *Ropalidia marginata*

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Abstract

We report here development and characterization of 48 novel microsatellite markers for *Ropalidia marginata*, a tropical, primitively eusocial polistine wasp from peninsular India. Thirty-two microsatellites showed polymorphism in a wild population of *R. marginata* (*N* = 38) collected from Bangalore, India. These markers will facilitate answering some interesting questions in ecology and evolutionary biology of this wasp, such as population structure, serial polygyny, intra-colony genetic relatedness and the pattern of queen succession.

Keywords: genetic variation, microsatellites, queen succession, *Ropalidia marginata*

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Ropalidia marginata (Lep.) (Hymenoptera: Vespidae) is a tropical polistine wasp, widely distributed in peninsular India (Gadagkar 2001). At a certain point of time, a nest consists of only one queen and several workers, although a nest can be under the regime of several queens during its existence — a phenomenon known as serial polygyny. This species is considered primitively eusocial, because of the absence of morphological differentiation between queens and workers and because many, if not all, female wasps can mate, develop their ovaries and function as solitary nest foundresses or as queens of multiple foundress nests (Gadagkar 2001). However, there is growing evidence that *R. marginata* is different from other primitively eusocial species. In all other primitively eusocial species studied so far, queens are the behaviourally most dominant and active individuals on the nest, and thus use physical aggression (dominance behaviour) to suppress worker reproduction. In contrast, *R. marginata* queens are described as behaviourally nondominant, docile sitters, who cannot possibly inhibit worker reproduction by physical aggression and who probably use pheromones to do so (Sumana & Gadagkar 2003). Because of the wealth of behavioural information already available on *R. marginata* (Kardile & Gadagkar 2003; Sumana & Gadagkar 2003; Agrahari & Gadagkar 2004; Bruyndonckx *et al*. 2006; Bhadra *et al*. 2007), the availability of molecular markers will further enhance our understanding of this species, especially the role of intracolony genetic relatedness in social evolution and the pattern of queen succession. Therefore, in the present study, we have developed informative microsatellite markers for *R. marginata*. These markers can also be employed in studying population genetic structure of this species.

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In order to develop microsatellite markers, repeat enriched genomic library was constructed using the following procedure. Genomic DNA was extracted from adults of *R. marginata* collected from the Indian Institute of Science campus, Bangalore, India. A pool of 20 adults from different nests was collected and used for DNA extraction. Hybridization capture approach (Glenn & Schable 2005) was employed for enriching the DNA pool with repeat harbouring fragments. In brief, genomic DNA was digested with the restriction enzyme *Rsa*1 (New England Biolabs, UK), and 300–1000-bp inserts were ligated into the super SNX double-stranded linker on both sides. The DNA was hybridized with biotinylated oligonucleotides such as $(ATT)_8$, $(ATGA)_7$, $(CA)_{10}$, $(GA)_{10}$, $(GATA)_7$ and $(CAC)_7$, and separated with Dynabeads (Dynal) under magnetic field. The products eluted from beads were polymerase chain reaction (PCR) amplified using the super SNX forward primer (GTTTAAGGCCTAGCTAGCAGAATC) and cloned into pCR2.1-TOPO vector (Invitrogen). Resulted recombinant plasmids were transformed into XL1-Blue competent cells. Plasmids were extracted from clones with more than 200-bp inserts, using QIAprep Spin Miniprep Kit (QIAGEN). The inserts were sequenced using BigDye terminator chemistry on an ABI 3100 automated sequencer (Applied Biosystems) with M13 primers.

Of the 415 clones of *R. marginata* microsatellite-enriched library, 148 were picked and their plasmid inserts were sequenced. The sequences were screened for the presence of microsatellites using the software Micas (www.cdfd.org.in/micas). Primers were designed for sequences flanking the

Table 1 Characteristics of *Ropalidia marginata* microsatellite loci genotyped on ABI PRISM 3730 DNA analyser, including repeat motif, primer sequences, annealing temperature, number of individuals screened (N), number of alleles (N_a), observed (H_O) and expected heterozygosities (H_E). Data obtained upon screening 38 individuals sampled from three locations of Bangalore region

Locus (GenBank)	Fluoerescent label	Colour	Repeat motif	Primer	Ann. temp.	N/N_a	Size range	H_O	H_E
RmSat006† (FJ589173)	TAM	Yellow	$(TAAAAAA)_3$	F: TACCGCAACATTTTATTTCA R: TCCTGTCTCAATACCGTTAGA	56 °C	36/5	266–274	0.194	0.603*
RmSat010† (FJ589175)	JOE	Green	$(CCA)_2A(CCA)_7(GCA)_2$ ... $(CT)_5$	F: GAATCTTCTCTCATCCACCA R: GTGTAGCATCCCTCGTAAAG	56 °C	32/10	132–145	0.531	0.791*
RmSat011† (FJ589176)	TAM	Yellow	$(TAAA)_9$	F: ATGCGTTCGAACACCTGAGCT R: CGAATTCCCTAGGCTTATAG	56 °C	36/8	226–247	0.361	0.749*
RmSat013 (FJ589178)	JOE	Green	$(TAA)_3TCA(TAA)_3TCA(TAA)_3$	F: TAGGAACGGAATCTACCAAC R: CAAATCGCATTTGTCAGTAA	58 °C	34/10	106–117	0.706	0.791*
RmSat019 (FJ589179)	FAM	Blue	$(CA)_5(CAAA)_2(CA)_2$ ... $(CATA)_2(CA)_{11}$	F: TCTCAGTTTCATTCGGATTT R: CTTTTCGTGATATCGACTGC	56 °C	32/14	233–256	0.781	0.895*
RmSat028† (FJ589182)	FAM	Blue	$(CT)_9(CTTT)_3$	F: GTCGACCCACAACCGATACT R: TCCTCCCCGGATATCACATA	58 °C	35/11	247–264	0.571	0.822*
RmSat065† (FJ589193)	TAM	Yellow	$(CTGA)_2(CTTA)_4$ ... $(CA)_{13}(CACG)_4$	F: TTCAAGGACAAACGCTATTT R: GTTTGAACGTAAAGGGAGTG	56 °C	35/16	162–186	0.543	0.928*
RmSat067† (FJ589194)	JOE	Green	$(CAA)_6$	F: CACTTCTCTTCCCACTTGAG R: CGTGGATAAAGATTGGTGAT	55 °C	35/6	221–228	0.0571	0.661*
RmSat074 (FJ589197)	TAM	Yellow	$(GTGA)_8$ ... $(GA)_5$	F: AGAGAAGGAAAGGGACAGAG R: CTAACAGCAACAGCACTCCT	56 °C	30/8	101–133	0.700	0.820
RmSat083† (FJ589201)	JOE	Green	$(AGT)_7$ ... $(AGT)_{10}$	F: GCCAACAGATCCAACAGTAA R: CCTGTTCCTACTGCTGCTAC	56 °C	37/16	170–191	0.514	0.893*

*Significant deviation from Hardy–Weinberg equilibrium. Loci marked (†) denote presence of null alleles.

microsatellites using Primer 3 software (Rozen & Skaletsky 2000). Each primer set was tested for proper amplification by PCR. Optimal annealing temperature for each set was obtained using a temperature gradient thermal cycler (Eppendorf GmbH).

Primers were designed for 84 repeat-containing loci and 48 were selected for subsequent screening for polymorphism. The others were rejected due to nonspecific amplification or absence of PCR product. Among these microsatellite loci, the majority of them were dinucleotide repeat motifs (66%) followed by tri- (26%) and tetranucleotide repeat motifs. A very low percentage of hexa- and heptanucleotide repeat motifs (2% each) were also identified in this study. Among the dinucleotide repeats, the GA repeat motif was the most common accounting for 69% followed by 27% of GT repeat motifs, suggesting that the GA/GT repeats are abundant in hymenoptera as reported by others (Krieger & Keller 1997; Gopinath *et al*. 2001; Paxton *et al*. 2003; Solignac *et al*. 2003; Arthofer *et al*. 2005). The length of dinucleotide repeats varied from five to 28, as compared to trinucleotide repeats which varied between four and 13. For the population genetic study, wasps sampled were preserved in 70% ethyl alcohol and DNA was extracted using HotSHOT (Truett *et al*. 2000) method at the earliest opportunity.

Fourteen microsatellite loci were confirmed to be monomorphic and 32 loci were found to be polymorphic. Two loci namely RmSat043 and RmSat070 were highly polymorphic but produced unscorable stutter bands.

Out of 32 polymorphic loci, 10 were evaluated using fluorescent-labelled primers on an ABI PRISM 3730 DNA analyser using DNA from 38 females sampled from three different localities across Bangalore, India (Table 1). The remaining 22 loci were evaluated on 12% PAGE using DNA from 38 female wasps sampled from another population collected from Bangalore (Table 2). The primers were labelled using three different fluorescent dyes, that is, TAM (Yellow), JOE (Green) and FAM (Blue). The PCR was performed on an Applied Biosystems, GeneAmp PCR System 9700. The amplification conditions include an initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 30 s, a locus-specific annealing temperature (Tables 1 and 2) and 72 °C for 45 s, followed by a final extension of 72 °C for 10 min. The 10 loci given in Table 1 were run on an ABI PRISM 3730 DNA analyser, with Pop-7 as sieving matrix, HiDi Formamide as single-stranded DNA stabilizer and GeneScan 500 ROX standard as a size marker. ABI PRISM GeneMapper software version 3.0 was used to size the alleles. The amplified products of the remaining 22 loci (Table 2) were separated on 12% polyacrylamide gels using Hoefer SE 600 standard vertical electrophoresis unit along with 50-bp DNA ladder (MBI Fermentas) and allelic products were visualized by silver staining. The alleles were scored using Bio-Rad Quantity One software (Bio-Rad Laboratories). Analyses of polymorphism, including number of alleles, observed heterozygosity, expected heterozygosity, likelihood ratio test for linkage disequilibrium and exact

Table 2 Characteristics of *Ropalidia marginata* microsatellite loci on polyacrylamide gel, including repeat motif, primer sequences, annealing temperature, number of individuals screened (N), number of alleles (N_a), observed (H_O) and expected heterozygosities (H_E). Data obtained upon screening 38 individuals sampled from a population collected from Bangalore region

Locus	Repeat motif	Primer	Ann. temp.	N/N_a	Size range	H_O	H_E
RmSat001 (FJ589170)	GCT(ACT)$_2$(GCT)$_{11}$	F: ATTTCGAACAAAAGCACCT R: AGGGATAACCGAAATGGA	58 °C	38/3	273–300	0.395	0.442
RmSat002† (FJ589171)	(CA)$_9$ ... (CAG)$_6$(CTG)$_2$	F: GCGACTCAATTTCTGTTTCT R: AAACCAATAACAGCAACCAC	58 °C	38/6	187–210	0.378	0.783*
RmSat005† (FJ589172)	(TA)$_5$	F: TAAAAAGGGATGGATGTGTC R: TCATCGGACAAACTGTGAG	58 °C	38/3	146–156	0.211	0.482*
RmSat007 (FJ589174)	(CA)$_5$ ... (GCACCA) (GAACCA)$_3$(GCACCA)	F: CAAGTGACATGTCGATGAGA R: AGTAAACGGAGCTACTGCTG	58 °C	38/2	217, 229	0.316	0.438
RmSat012† (FJ589177)	(CGT)$_2$TGT(CGT)$_{13}$	F: TGTTATCAAGTGACGAAACG R: CGTTGTTACATCGTTCAAAG	58 °C	37/7	113–149	0.605	0.760*
RmSat020† (FJ589180)	(CA)$_4$AG(CA)$_5$TA(CA)$_4$GA(CA)$_3$	F: CGATCTAACTCTGTGGCTCT R: ACGTTCAAGTTGAGTCCAAT	58 °C	38/4	122–136	0.447	0.699*
RmSat022† (FJ589181)	(CA)$_{19}$	F: CGATTAAACGTATTCATTATGG R: GTTGTTGCTCTCGAAAAACT	56 °C	38/5	180–204	0.500	0.737*
RmSat029 (FJ589183)	(GA)$_2$AA(GA)$_4$AA(GA)$_3$ AA(GA)$_3$AG(GA)$_2$	F: GTATTGCATCGTGTGTTTGT R: AGAGCCACAGAGTTAGATCG	58 °C	38/2	117, 125	0.026	0.026
RmSat030 (FJ589184)	(GA)$_2$AA(GA)$_{20}$	F: TCCCGTAGTTCCTATCAATG R: TTCATACACGTTTTCATCCA	54 °C	38/5	136–154	1.000	0.771*
RmSat036 (FJ589185)	(CT)$_6$TT(CT)$_2$	F: ACCAATAATACACACGCAGA R: TCGTATTTTCTCCCTCGATA	56 °C	38/2	222, 230	0.342	0.287
RmSat039 (FJ589186)	(GA)$_{10}$	F: ATATGGGTTCTCGATGAAAG R: ACATGAAAGACACACACGAA	56 °C	38/5	114–128	0.447	0.559*
RmSat041 (FJ589187)	(CT)$_7$C(CCTT)$_2$T(CT)$_5$	F: ACAACGCAATCTCCGTATTA R: GTTCAAGACGGTGAAAAATG	56 °C	38/2	165, 177	0.158	0.147
RmSat045† (FJ589188)	(GA)$_2$AA(GA)$_9$AA(GA)$_3$A(GA)$_2$	F: CATAACTTCTACGGCCTTGT R: TAACGTTTCGCGATAAAGAC	56 °C	38/7	143–170	0.526	0.735*
RmSat047 (FJ589189)	(GGA)$_2$(GA)$_3$AA(GA)$_9$	F: GATCGTCCCATACTCAGAGA R: CAGACCGGGAGAATTTCTAT	56 °C	37/10	136–180	0.730	0.861*
RmSat049 (FJ589190)	(CT)$_8$ ... (CT)$_{10}$	F: TCACATAGACGCAAACACAT R: AGTCTAGGATTGAGGGGAAG	56 °C	38/2	165 171	0.026	0.026
RmSat056 (FJ589191)	(GA)$_{14}$	F: CGAACCATTCCACCACATAC R: CCGCGACCAACCAAATAC	58 °C	38/8	88–132	0.789	0.837
RmSat064† (FJ589192)	(GT)$_{10}$AT(GT)$_4$	F: AAAGAAGTTCGAGCCAAAG R: ATTACAATTGCGAGCAAACT	54 °C	38/6	265–312	0.500	0.755*
RmSat071† (FJ589195)	(CT)$_4$CA(CT)$_{13}$TT(CT)$_3$	F: CCATCACCGAAGAAACTAAT R: AGAAGACGTAGATACCCCAAC	55 °C	38/10	139–185	0.632	0.838*
RmSat072 (FJ589196)	(CT)$_{11}$	F: CGACTAACTTCATCGTTTCC R: ACGCAACGATGTTTAATTTT	56 °C	38/2	147, 153	0.079	0.077
RmSat077† (FJ589198)	(GA)$_7$A(GAG)$_3$	F: CTCTAAACCCCGATAATGTTT R: AACTAAACGGATGAAACGAA	56 °C	38/4	116–131	0.184	0.689*
RmSat079 (FJ589199)	(CT)$_9$	F: CTGGTGCTAAGTGAAAATCC R: ATACGTGCGCGTAAATAAAT	56 °C	38/4	172–184	0.658	0.529
RmSat082 (FJ589200)	(CAA)$_6$(CAG)$_3$(CAA)$_2$	F: TGTCCCACAGTATCCTCAAT R: AATGACAGACTTTTGCGACT	56 °C	38/2	196, 202	0.053	0.052

*Significant deviation from Hardy–Weinberg equilibrium. Loci marked (†) denote presence of null alleles. Allele sizes for these loci may not be accurate as these loci were resolved on polyacrylamide gels.

tests for the Hardy–Weinberg equilibrium were performed by using Arlequin version 3.1 (Excoffier *et al*. 2005). The probability of occurrence of null alleles was tested using Micro-Checker (van Oosterhout *et al*. 2004).

The number of alleles per locus ranged from two to 16, and the observed and expected heterozygosities ranged from 0.026 to 0.895. After sequential Bonferroni correction, 35 of the 45 combinations showed significant linkage disequilibrium in individuals scored on automatic DNA analyser. However, in case of the loci analysed on PAGE, none of the 231 pairwise comparisons between loci showed significant linkage disequilibrium. Significant deviations ($P < 0.05$) from Hardy–Weinberg equilibrium were detected in 21 out of 32 loci (Tables 1 and 2). Departure from

Hardy–Weinberg equilibrium was probably due to heterozygote deficit. However, this could be interpreted as the result of Wahlund effect (Wahlund 1928), because the Bangalore population may have been structured into several subpopulations. Null alleles may be present in 16 of the 32 loci reported here. The informative microsatellite markers reported in the present study will be of great value for answering interesting questions in ecology and evolutionary biology of *R. marginata*.

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Development and optimization of sequence-tagged microsatellite site markers to detect genetic diversity within *Colletotrichum capsici*, a causal agent of chilli pepper anthracnose disease

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Abstract

Genomic libraries enriched for microsatellites from *Colletotrichum capsici*, one of the major causal agents of anthracnose disease in chilli pepper (*Capsicum* spp.), were developed

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