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PRIMER NOTE

Microsatellite loci for genetic research in the hornet *Vespa mandarinia* and related species

EISUKE HASEGAWA* and JYUNICHI TAKAHASHI†

**Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido, University, Kita-ku, Sapporo 060–8589, Japan, †Laboratory of Systematic Entomology, Department of Ecology and Systematics, Graduate School of Hokkaido University, Kita-ku, Sapporo 060–8589, Japan*

Abstract

We developed 5 microsatelitte loci in the hornet *Vespa mandarinia* from RAPD fragments. The 5 loci showed 3–7 alleles in *V. mandarinia* and many were also polymorphic in the related species, *V. ducalis*, *V. analis*, *Dolichovespula norvegicoides* and *Vespula schrenckii*. These results suggested the loci will be useful for analyzing genetic structure of Vespinae, at both the colony and population levels.

Keywords: Hornet, Microsatellite DNA, *Vespa mandarinia*, *Vespula*, *Dolichovespula*

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Hornets of the genus Vespa (subfamily Vespinae) are distributed widely from tropical to temperate regions, with 23 described species (Archer 1991). All species in the subfamily are highly eusocial, but members of the genus vary remarkably in social behaviour (Matsuura & Yamane 1990). Studies using microsatellites can provide valuable information used to understand social evolution in the Vespinae, as has been seen in other social Hymenoptera (Evans 1993; Hughes & Queller 1993; Estoup *et al*. 1994), although only a few characterized loci exist for this family (Thoren *et al*. 1995). In this article we describe five variable microsatellite loci developed in *V. mandarinia* and cross-species tests in four related species, *V. analis*, *V. ducalis*, *Vespula schrenckii*, and *Dolichovespula norvegicoides*.

We developed microsatellite loci from random amplified polymorphic DNA (RAPD) fragments. The DNeasy Tissue Kit (QIAGEN) was used to extract total DNA from part of a thoracic muscle of an acetone preserved sample of *V. mandarinia*. The total DNA was randomly amplified using a 10 bp long primer of random nucleotide sequence (Operon Technologies Inc.) in a PTC-100 thermal cycler (MJ research Inc.). A total of 40 primers (OPA01-20, OPB01-20) was used in this study. Each RAPD reaction (15 μL) contained 15 ng template DNA, 1.5 μL of 10× reaction buffer, 1.5 μL of dNTP mix (1 mM of each dNTP), 18 pmol of one of 20 primers, and 0.6 unit of *Taq* DNA polymerase (TaKaRa Taq, TaKaRa). The thermal cycle profile consisted of 45 cycles of 1 min at 94 °C, 1 min at 35 °C, a 0.3 °C/ s temperature transition to 72 °C and 2 min at 72 °C. One microliter of product from each different primer was dropped separately on a positively charged nylon membrane (FMC). After drying at room temperature, DNAs on the membrane were blotted by normal alkaline transfer.

RAPD products containing microsatellite regions were detected using a biotinylated DNA detection kit (Imaging High -Color-, TOYOBO) and biotin-labelled oligonucleotides [$(GA)_{10}$ $(AT)_{10}$ $(GC)_{10}$ $(AGC)_7$ $(AGT)_7$ $(ACG)_7$ and $(ATG)_7$] as probes for microsatellites. Nineteen of 40 primers' products were positive. The remaining products from each positive primer were mixed together and were purified by QIamp PCR purification Kit (QIAGEN) in 10 mL of the final volume. Both ends of the purified fragments were blunted, kinased using TaKaRa BKL Kit (TaKaRa). The treated fragments were ligated into *Sma*I-cut pUC19 plasmids using the same kit, following the manufacturer's instructions. Recombinant plasmids were transformed into competent *Escherichia coli* cells (Competent High -JM109-, TOYOBO).

A total of 75 recombinant colonies were picked and were suspended independently in 20 μL of TE [10mM Tris-Cl (pH7.4), 1mM EDTA (pH8.0)]. Each suspended colony was boiled at 98 °C for 2 min and then centrifuged 1 min at 18 600 *g*. Inserts were amplified by a polymerase chain

Correspondence: Eisuke Hasegawa. Fax: + 81-11-757-5595; E-mail: ehase@res.agr.hokudai.ac.jp

Table 1 Primers, PCR conditions and core sequence of 5 *Vespa mandarinia* microsatellites

Locus name	Core sequence in cloned alleles	Prime sequence (5′–3′)	$MgCl_2$ (mM)	Annealing temp (°C)	Cycles	Allele size (bp)	GenBank Accession No.
VMA-3	$(GA)_{19}\ldots(CT)_{14}$	f: 5′-ATA CCG CGA AAG AGT TTC G-3′	1.5	52	30	257	AB060626
		(r) 5′-TAA TTT GAG GCA GGC TAA T-3′					AB060627
VMA-4	$(GA)_{13}$	f: 5′-ATG TAA AAA TTG CGC ACG TT-3′	1.5	54	30	306	AB060628
		(r) 5′-ACG CGA AGA TAG CGA CAA CG-3′					AB060629
VMA-6	$(CT)_{16}$	f: 5′-ACA GTT TCT TGA TTC GTC G-3′	1.5	56	30	252	AB060630
		(r) 5′-GAT GCT ATC GTC GGC ATT T-3′					AB060631
VMA-7	$(CT)_{19}$	f: 5′-GCT GTC GAG TGT GCG TTT A-3′	1.5	58	30	188	AB060632
		(r) 5′-TGT TTG GAA AGC GAA CGA C-3′					AB060633
VMA-8	$(CT)_9$	f: 5′-TAG ACA CGT ACA CCA CTA G-3′	1.5	58	30	113	AB060634
		r: 5′-CTG GCC AGG ATA TTC CAG T-3′					AB060635

Table 2 Results of cross-species application tests of the microsatellite loci from *Vespa mandalinia* to four related species. Number of alleles (N_A), expected heterozygosity (H_E) and the observed heterozygosity (H_O) are shown for each species

	Vespa mandarinia (n = 20)			*Vespa ducalis* (n = 20)			*Vespa analis* (n = 20)			*Dolichovespula norvegicoides* (n = 20)			*Vespula schrenckii* (n = 20)		
Locus name	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O	N_A	H_E	H_O
VMA-3	5	0.72	0.75	4	0.72	0.75	3	0.59	0.65	3	0.53	0.60	4	0.63	0.75
VMA-4	3	0.53	0.65	2	0.10	0.10	2	0.18	0.20	1	0.00	0.00	1	0.00	0.00
VMA-6	4	0.68	0.70	3	0.44	0.50	2	0.41	0.45	3	0.53	0.60	3	0.60	0.65
VMA-7	7	0.75	0.85	4	0.67	0.70	4	0.54	0.60	3	0.44	0.50	4	0.63	0.70
VMA-8	4	0.57	0.60	4	0.54	0.65	3	0.47	0.50	3	0.53	0.60	3	0.54	0.60

reaction (PCR) using a primer pair for the multicloning site of pUC19 (BcaBESTTM Sequencing Primer RV-M and M13-47, TaKaRa). Each 25 μL of PCR reaction contained 2.5 μL of 10× reaction buffer, 2.5 μL of dNTP mix (1 mM of each dNTP), each 0.5 μL of primers (10 pmol/mL), 0.2 U of TaKaRa Taq (TaKaRa), 2.5 μL of DNA (about 20 ng) and 16.4 μL of sterlized ddW. Temperature cycles were as follows; 30 cycles of 1 min at 94 °C; 1 min at 53 °C; 3 min at 70 °C. After PCR, 1 μL of each amplified product was blotted on a nylon membrane (FMC), and clones containing microsatellite sequences were detected by the same procedure presented above. Twenty-five of 75 clones were positive. For positive clones, 4 μL of the remaining PCR product was checked for its length on 1% agarose gel in TBE. There are 8 unique insert lengths (500 bp–1300 bp). The remaining product of each positive clone was purified by QIamp PCR Purification Kit (QIAGEN) and sequenced using an automated sequencer (ABI 373S, Applied Biosystems).

To test effectiveness of the obtained microsatellite loci, we checked amplification and polymorphism of the primer pairs (Table 1) in *V. mandarinia* and four related species including two other genera (Table 2). Total DNA was extracted from muscular tissues of thorax of individual samples by boiling grounded tissue in 300 μL of 5% Chelex resin (Bio-Rad) for 2 h at 55 °C (Walsh *et al*. 1991). PCR amplifications were carried out in a total volume of 10 μL, which contained 1.5 μL (*c*. 10 ng) of template DNA, 2.5 pmol of each microsatellite primer, 400 mM of dNTP, 1 μL of 10× reaction buffer, 1.5 mM $MgCl_2$ and 0.05 U of TaKaRa Taq (TaKaRa). All PCR reactions were performed using a GeneAmp 2000 (Perkin Elmer) thermocycler. Temperature cycles were as follows: 3 min at 94 °C followed by 30 cycles of 30 s at 94 °C; 30 s at 52–58 °C (for annealing temperature of each primer pair, see Table 1); and 30 s at 72 °C. PCR products were run on polyacrylamide gels (Bio-Rad) and visualized by silver staining kits (FMC).

Five of eight primer pairs were polymorphic with two to seven alleles in the three species of *Vespa* (Table 2). One locus (VMA-4) showed monomorphism in *Vespula schrenckii* and *Dolichovespula norvegicoides*, but the other four loci were polymorphic (three to four alleles) in these species. The number of alleles and levels of expected heterozygosity of each locus ranged from with two to seven and from 0.18 to 0.75, respectively (Table 2). Thus, we concluded that these microsatellite markers will be useful in genetic analysis on both population and colony level in species of Vespinae.

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