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POLYMORPHIC MICROSATELLITE LOCI FOR THE PRIMITIVELY EUSOCIAL WASP *ROPALIDIA FASCIATA* (HYMENOPTERA: VESPIDAE)

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Summary. A polymorphic microsatellite locus was isolated and characterized from *Ropalidia fasciata* (Fabricius, 1804), one of the most common in South-East Asia independent-founding polistine wasps (Vespidae: Polistinae). Four other microsatellite loci for which the primer sets were originally developed in two *Ropalidia* species also showed polymorphism in the size of amplification products in *R. fasciata*.

Key words: Vespidae, Polistinae, *Ropalidia*, polistine wasps, DNA, Japan.

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Резюме. Полиморфный микросателлитный локус выделен и охарактеризован у *Ropalidia fasciata* (Fabricius, 1804) – одного из наиболее распространенных в Юго-Восточной Азии видов ос-полист (Vespidae: Polistinae). Четыре других микросателлитных локуса, для которых первоначально были разработаны наборы праймеров у двух видов рода *Ropalidia*, также показали полиморфизм в размере продуктов амплификации у *R. fasciata*.

INTRODUCTION

Ropalidia fasciata (Fabricius, 1804) (Vespidae: Polistinae) is a primitively eusocial wasp that is common in South-east Asia including the islands of Okinawa, Japan. Because single- and multiple-foundress colonies of this species are co-existing from the nest initiation to the emergence of the first workers, great interests have been paid by many researchers. For example, Itô (1983a,b, 1985) observed in this species that the dominance-subordination relation

among the co-foundresses are milder than those reported for *Polistes*. Thus, he proposed the communal aggregation hypothesis to explain the maintenance of multiple-foundress colonies and an evolutionary pathway from primitively eusocial to polygynous eusocial societies in polistine wasps (Itô, 1993). On the other hand, Iwahashi (1989) observed monopolization of egg-laying by a dominant foundress of this species and suggested that subordinate foundresses increase the indirect fitness through a genetically related dominant foundress. However, to address those issues, it is needed to know colony genetic structure of pre-emergence, multiple-foundress colonies, particularly information about relatedness between co-foundresses, and maternity of immature brood. The present short note aims to describe a highly polymorphic microsatellite primer set for the independent-founding wasp *R. fasciata*.

MATERIAL AND METHODS

Total DNA was extracted from about 50 individuals (adults and larvae, about 1.0 g) in a well-developed colony collected in Okinawa in autumn 1994. They were homogenized in 5 ml of lysis buffer (100 mM NaCl, 100 mM Tris-HCl pH 8.0, 100 mM EDTA), and added to 600 µl of 10 % SDS, 200 µl of 10 mg/ml protainase K (WAKO), and 50µl of 5 mg/ml RNase A (Boehinger Manheim). The mixture was incubated at 55℃ overnight. After three phenol (saturated with 100 mM Tris-HC (pH 8.0)), one phenol-chloroform, and one chloroform-isoamyl alcohol extraction, DNA was precipitated with two volume ethanol and 1/10 volume 3M CH3COONa (pH 5.2). The samples were incubated at –20℃ for 20 min and then centrifuged for 10 min at 11200×g in room temperatures. The DNA pellet was rinsed twice with chilled (–20℃) 70% ethanol before dried up. DNA was resuspended in 500 µl of TE buffer (1 mM EDTA, 10 mM Tris-HCl (pH8.0) and kept at 4℃. The DNA was quantified with a fluorometer (HITACHI, U-3210). On this step, a total of high molecular DNA was about 120µg. This DNA was digested with a restriction enzyme EcoR1 (TOYOBO) at 37℃ overnight. The digested DNA was electrophoresed in a 1% agarose (1×TBE buffer) gel. Fragments in the size range 100-1000 bp were recovered by using DEAE membrane. The recovered fragments were cloned into lambda gt10 vector (STRATAGENE). After plaque hybridization (the probe was a synthesized oligonucleotide of 10 times repeats GT), positive plaques were picked up, and the cloned fragment was sequenced by an auto sequencer (ABI, 373A). Six primer sets were designed for the flanking regions surrounding the microsatellites (RF1, RF2, RF3, RF4, RF5, RF6). However, only one (RF2) of the 6 primer sets successfully produced amplification products showing polymorphisms in size. The sequence, including tandem repetitive regions, has been deposited in DDBJ under Accession nos LC484426 for loci RF1 and LC495918-LC495922 for loci RF2-RF6.

Next, we try to find polymorphic microsatellite loci for which the primer sets were originally developed in other *Ropalidia* species. Genomic DNA of *R. fasciata* was extracted from a hind leg. The hind leg was homogeneized with pestles in 1.5 ml tubes, and then mixed with 50 µl extraction buffer containing 150 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1.0 mM EDTA, 10 µg proteinase K, and 40 µg Chelex 100 (Bio-Rad Laboratories). Samples were kept at 56°C for 2 h and at 99.9°C for 3 min (Walsh *et al*., 1991). Each solution was then precipitated with ethanol and maintained in TE buffer at 4°C. PCR was performed using 1 µl diluted genomic DNA (≈1ng) in a mixture consisting of 1 µl primer mix (2.5 mM), 0.1 µl of 10 mM dNTP mix, 0.05 µl *Taq* polymerase (5 U/ml Takara *Ex Taq*; Takara Bio, Inc.), 1 µl of 10× PCR buffer (provided with the polymerase, containing 1.5 mM $MgCl_2$), and 6.85 µl dH_2O in a total volume of 10 µl. PCR was carried out using a thermal cycler (Gene Amp PCR

Table 1. Microsatellite loci for *Ropalidia fasciata*

Locus	Cloned core sequence	Primer sequence (5'-3')	Size (bp)	Annealing temperature (°C)	Cycles	Allele no.	He	Ho	References
RF2	$(GA)_{12}A$ $AA(GT)_{25}$	F: CCGTTATGAGAAAGAAGTAT R: CGTAATCCTCTTGTTTAAGA	230	50	26	8	0.787	0.798	This study
Rrev128	$(AAT)_{15}$	F: GGTTAGCACAAGCAAGGACAA	157	50	28	6	0.661	0.745	Henshaw *et al.*, 2003
		R: CCATAGAAATCGTTGACAAGG							
Rrev188	$(AG)_{15}$	F: ATTCAAGGGCTGTTCTCAGTC	123	50	26	3	0.409	0.435	Henshaw *et al.*, 2003
		R: GGGCGATAAAGAAAGGAAAAG							
Rrev305	$(AAG)_7$	F: TGCGAGTAACCGGCAAAGA	116	50	27	3	0.553	0.506	Henshaw *et al.*, 2003
		R: TATGGCCAACTGCGTTCCTAT							
Rsnv446	$(CTTT)_3$ $(CCTT)_5$	F: ACCTTGAGCCAAGTTACCGAA R: TTGGAAGTCACGGTGAGAAA	151	50	28	3	0.609	0.718	Henshaw *et al.*, 2003

He: expected heterozygosity, Ho: observed heterozygosity.

System 2700; Applied Biosystems) programmed for an initial denaturation for 4 min at 94°C, followed by 24–35 cycles of 1 min at 94°C, 1 min at the annealing temperature of each primer set (described in the Table 1), and 45 s at 72°C, with a final extension for 7 min at 72°C. The PCR products obtained were electrophoresed in 8% polyacrylamide gels and visualized by silver staining (Bassam *et al*., 1991). Genotype scoring and data entry were conducted independently by two people, and their scores were compared. Discrepancies were rechecked and if necessary, the sample was rerun. Statistical analyses were carried out using the program package Arlequin ver. 3.11 (Excoffier *et al*., 2005).

RESULTS AND DISCUSSION

We found that four microsatellite loci (Rrev128, Prev188, Prev305, Rsnv446) for which the primer sets were originally developed in two *Ropalidia* species (Henshaw *et al*., 2003), show polymorphisms in size in *R. fasciata*. The PCR conditions for a total of five primer sets are given in Table 1. The number of alleles per locus ranged from three to eight with a mean of 4.6, and the observed heterozygosity ranged from 0.435 to 0.798, with a mean of 0.640 (Table 1). In all five loci, we successfully detected PCR products for all males (N=134) and therefore null alleles could be ruled out. Each locus was tested for Hardy-Weinberg equilibrium, but none of all loci did not significantly deviate. Each locus was also tested for genotyping linkage disequilibrium using Arlequin, where we randomly selected one adult female from each colony for calculation. None of the tested loci showed significant linkage disequilibrium (all *P*-values > 0.05 after Bonferroni correction for multiple comparisons). The five microsatellite loci provide a powerful means to examine the social and genetic structure of *R. fasciata* colonies and will enhance our ability to answer important questions regarding genetic conflicts of interest among nestmate individuals.

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