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Sociobiology 63(2): 841-844 (June, 2016) DOI: 10.13102/sociobiology.v63i2.899

Sociobiology

An international journal on social insects

SHORT NOTE

Polymorphic Microsatellite Loci in the Independent-founding Wasp *Polistes versicolor* (Hymenoptera: Vespidae)

Y Takahashi[1], A Yamada[1], S Mateus[2], R. Zucchi[2], FS Nascimento[2], K Kudô[1]

1 - Niigata University, Niigata, Japan

2 - Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Ribeirão Preto-SP, Brazil

Article History

Edited by
Gilberto M. M. Santos, UEFS, Brazil
Received 17 August 2015
Initial acceptance 07 April 2016
Final acceptance 26 April 2016
Publication date 15 July 2016

Keywords

Corresponding author
Kazuyuki Kudô
Laboratory of Insect Ecology, Faculty of Education, Niigata University
Niigata, 950-2181 - Japan
E-Mail: kudok@ed.niigata-u.ac.jp

Abstract

Microsatellite primers developed for a given species are sometimes useful for another in the same genus, making possible to search for pre-existing suitable primers in the data banks such as GenBank. We examined whether existing primers developed for *Polistes* wasps could be used for the independent-founding wasp *Polistes versicolor*. We tested 50 microsatellite primers from three *Polistes* species and found that five microsatellite loci show polymorphism in size in *P. versicolor*. These five loci were highly polymorphic, having four to 10 alleles in *P. versicolor* with an expected heterozygosity of 0.530–0.836. These loci can be used to study parameters concerning genetic relatedness such as social interactions in colonies and genetic conflicts of interest among nestmate individuals.

In recent years, many microsatellite primers have been developed for various animals and plants and the sequences of these primers have been deposited in data banks such as GenBank. Microsatellite primers developed for a given species are sometimes useful for other species in the same genus (Henshaw et al., 2001; Kudô et al., 2005; Katada et al., 2007; Komatsu et al., 2012), so it is possible to search for pre-existing suitable primers in the data banks.

Polistes versicolor Olivier is an independent-founding wasp that builds nests formed by a single, uncovered comb attached to the substratum by a single petiole (Richards, 1978). In this species, colonies show different development stages throughout the year and are influenced by climatic and ethological factors (Gobbi & Zucchi, 1980, 1985), which characterize an asynchronous biological cycle. Strassmann et al. (1989) and Junior et al. (2010) estimated genetic relatedness among nestmates in *P. versicolor* using enzymes, but no study has been made to evaluate colony genetic structure in this species using microsatellite markers. In this study, we examined whether existing primers developed for *Polistes* could be used for *P. versicolor*.

Eleven colonies of *P. versicolor* were collected at a farm near the town of Cajuru (47º15’S; 21º26’W) in the state of São Paulo, Brazil, in late March 2011. All insect samples were kept in 99.5% ethanol until DNA extraction.

We tested 50 microsatellite primers from three *Polistes* species, 22 from *Polistes chinensis antennalis* (F.) (Tsuchida et al., 2003, Saigo & Tsuchida, 2010), 10 from *Polistes rothneyi* (Cameron) (Takahashi & Yamasaki, 2007), and 18 from *Polistes bellicosus* (Cresson) (Strassmann et al, 1997), using 222 females of *P. versicolor* from 11 colonies (Mean ± SE: 20.18 ± 5.58) (Table 1). Genomic DNA of *P. versicolor* was extracted from two hind legs. The hind legs were 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

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ISSN: 0361-6525

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 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).

We found that five microsatellite loci for which the primer sets were originally developed in independent-founding paper wasps, *P. chinensis antennalis* (Pc25 and Pc76), *P. bellicosus* (Pbe411) and *P. rothneyi* (Pr4 and Pr10), show polymorphism in size in *P. vericollor* (Genbank Accesion Numbers, Pc25: AB190311; Pc76: AB190314; Pbe411: AF120410; Pr4: AB281175; Pr10: AB281181).

The number of alleles per locus ranged from four to ten with a mean of 6.6, and the observed heterozygosity ranged from 0.593 to 0.743, with a mean of 0.661 (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 deviated. 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 *P. versicolor* colonies and will enhance our ability to answer important questions regarding genetic conflicts of interest among nestmate individuals.

Acknowledgments

The authors thank J. M. Carpenter for the identification of the species. We also thank K. Komatsu and Y. Yamaguchi for their kind help during the field collection. This study was supported in part by a Grant-in-Aid for Young Scientists (B) (no. 21770017) to K. Kudô.

Table 1 - Characteristics of five microsatellite loci in *Poliste versicolo*r.

Locus	Size (bp)	Annealing temperature (C)	Cycles	Number of alleles	*He*	*Ho*	References
Pc25	185	50	28	4	0.737	0.743	Tsuchida et al., 2003
Pc76	223	50	28	7	0.584	0.608	Saigo & Tsuchida, 2010
Pbe411	173	50	28	4	0.530	0.593	Strassmann et al., 1997
Pr4	211	50	28	8	0.736	0.635	Takahashi & Yamasaki, 2007
Pr10	206	50	28	10	0.836	0.725	Takahashi & Yamasaki, 2007

He: expected heterozygosity; Ho: observed heterozygosity

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