



## SHORT NOTE

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

Y TAKAHASHI<sup>1</sup>, A YAMADA<sup>1</sup>, S MATEUS<sup>2</sup>, R. ZUCCHI<sup>2</sup>, FS NASCIMENTO<sup>2</sup>, K KUDÔ<sup>1</sup>

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 (47°15'S; 21°26'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 homogenized 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<sub>2</sub>), and 6.85 µl dH<sub>2</sub>O 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. versicolor* (Genbank Accession 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 versicolor*.

Locus	Size (bp)	Annealing		Number of alleles	<i>He</i>	<i>Ho</i>	References
		temperature (C)	Cycles				
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

### References

- Bassam, B. J., Caetano-Anolles, G. & Gresshoff, P. M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, 196: 80-83.
- Excoffier, L. G., Laval, G. & Schneider, S. (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online*, 1: 47-50.
- Gobbi, N. & Zucchi, R. (1980). On the ecology of *Polistes versicolor versicolor* (Olivier) in southern Brazil (Hymenoptera, Vespidae, Polistini). I. Phenological account. *Naturalia*, 5: 97-104.
- Gobbi, N. & Zucchi, R. (1985). On the ecology of *Polistes versicolor versicolor* (Olivier) in southern Brazil (Hymenoptera, Vespidae, Polistini). II. Colonial productivity. *Naturalia*, 10: 21-25.
- Henshaw, M. H., Strassmann, J. E. & Queller, D. C. (2001). Swarm-founding in the polistine wasps: the importance of finding many microsatellite loci in studies of adaptation. *Molecular Ecology*, 10: 185-191. doi: 10.1046/j.1365-294X.2001.01172.x.
- Junior, K. N., Simokomaki, K., Gruber, C. V. & Del Lama, M. A. (2010). Sociogenetic structure of *Polistes (Aphanilopterus) versicolor* Olivier, 1791 colonies (Hymenoptera, Vespidae, Polistini). *Genetics and Molecular Biology*, 33: 669-675. doi: 10.1590/S1415-47572010005000081.
- Katada, S., Suzuki, T. & Tsuchida, K. (2007). Application of microsatellite primers for the social wasp *Polistes* to another social wasp genus, *Parapolybia*, to estimate genetic relationships among nestmates. *Entomological Science*, 10: 1-5. doi: 10.1111/j.1479-8298.2006.00192.x.
- Komatsu, K., Mateus, S., Zucchi, R., Nascimento, F. S. & Kudô, K. (2012). Application of microsatellite primers developed for *Polistes* in the independent-founding wasp *Polistes satan*

- Bequaert (Hymenoptera: Vespidae). Neotropical Entomology, 41: 204-206. doi: 10.1007/s13744-012-0035-y.
- Kudô, K., Ishiguro, N., Tsuchida, K., Tsujita, S., Yamane, Sô. & Zucchi, R. (2005). Polymorphic microsatellite loci for the swarm-founding wasp *Polybia paulista* (Hymenoptera: Vespidae). Entomological Science, 8: 5-7. doi: 10.1111/j.1479-8298.2005.00093.x.
- Richards, O. W. (1978). The social wasps of the Americas, excluding the Vespidae. London: British Museum (Natural History) 580pp.
- Saigo, T. & Tsuchida, K. (2010). Nine newly designed polymorphic microsatellite loci for the Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). Applied Entomology and Zoology, 45: 575-577. doi: 10.1303/aez.2010.575.
- Strassmann, J. E., Hughes, C. R., Queller, D. C., Turillazzi, S., Cervo, R., Davis, S. K. Goodnight, K. F. (1989). Genetic relatedness in primitively eusocial wasps. Science, 342: 268-270.
- Strassmann, J. E., Barefield, K., Solís, C. R., Hughes, C. R. & Queller, D. C. (1997). Trinucleotide microsatellite loci for a social wasp, *Polistes*. Molecular Ecology, 6: 97-100.
- Takahashi, J. & Yamasaki, K. (2007). Isolation and characterization of 10 microsatellite loci in the paper wasp *Polistes rothneyi* (Hymenoptera; Vespidae). Molecular Ecology Notes, 7: 821-823. doi: 10.1111/j.1471-8286.2007.01715.x.
- Tsuchida, K., Saigo, T., Tsujita, S., Takeuchi, K., Ito, N. & Sugiyama, M. (2003). Polymorphic microsatellite loci for the Japanese paper wasp, *Polistes chinensis antennalis* (Hymenoptera: Vespidae). Molecular Ecology Notes, 3: 384-386. doi: 10.1046/j.1471-8286.2003.00457.x.
- Walsh, S. P., Metzger, D. A. & Higuchi, R. (1991). Chelex100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques, 10: 506-513.

