

PRIMER NOTE

Highly polymorphic microsatellite markers developed for the social halictine bee *Lasioglossum (Chilalictus) hemichalceum*

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Abstract

Lasioglossum (Chilalictus) hemichalceum is a social halictine bee species for which we developed 10 polymorphic microsatellite loci in order to investigate detailed genetic structure of cooperating individuals. The loci are highly polymorphic with allele numbers ranging between eight and 22. A null allele was detected at one locus in the absence of pedigree information.

Keywords: communal, Halictidae, Hymenoptera, microsatellite, null allele, sociality

Received 15 May 2002; revision received 26 July 2002; accepted 26 July 2002

Lasioglossum (Chilalictus) hemichalceum is a halictine bee endemic to Australia with 'communal' sociality (Kukuk *et al.* 1998; Ward & Kukuk 1998). Adult females live in small cooperative groups wherein all females reproduce. Group members are not relatives (Kukuk & Sage 1994) and this species is a model for the study of cooperation among unrelated individuals. To obtain more detailed information on the genetic structure of colonies, we undertook to develop microsatellite markers for this species. In addition, the Australian *Lasioglossum* consists of eight subgenera (Walker 1995) so that these loci may be useful for additional Australian *Lasioglossum*.

To generate markers, pupae were frozen in liquid nitrogen and pulverized in a 1.5 mL tube with a sterile pestle. Genomic DNA was extracted as described in Hamaguchi & Ito (1993). Small insert libraries were generated from the isolated genomic DNA and enriched for selected repeats (see Ostrander *et al.* 1992; Polido & Duyk 1994). Plasmid DNA prepared from clones from the small insert library were sequenced using an ABI 373. Polymerase chain reaction (PCR) primers for inserts of clones identified to contain repeats of significant length were designed using Designer PCR™ (Research Genetics, Division of Invitrogen Corp.

www.resgen.com). Primers pairs were synthesized (Research Genetics) with one of the primers containing a 5' HEX or FAM label.

Ten loci were successfully amplified using the PCR and showed variation. To extract DNA, the thorax of each adult was crushed, suspended in 200 µL H₂O containing 5% w/v Chelex resin beads (Bio101, Inc.), vortexed, autoclaved for 5 min, cooled, and centrifuged at high-speed to pellet the cell debris and resin beads. PCR reactions used 2 µL of the resulting supernatant, 1 × reaction buffer (Perkin-Elmer), 2.0 mM MgCl₂, 200 µM each dNTP, 2.0 µg/mL BSA, 0.4 µM each unlabelled primer, 0.2 µM labelled primer, and 0.4 U *Taq* polymerase (Perkin-Elmer) for a total volume of 10 µL. The PCR program was 94 °C/3 min, 50 °C/1 min (92 °C/1 min, 50 °C/1 min) × 34 cycles (Table 1). The PCR products were loaded on a 6% denaturing polyacrylamide sequencing gel and visualized using an FMBIO-101 gel scanner (Hitachi, Inc.). These loci were used to genotype 231 individuals from nine *L. hemichalceum* colonies (see Table 1).

Analysis of F-statistics and proportions of blank genotypes revealed that LHMS14 had a nonamplifying ('null PCR') allele. LHMS14 had a significant deviation from expectation when averaged over colonies ($F_{IS} = 0.231$; $P < 0.001$) and the proportion of blank genotypes was higher. In males it was 9/51 (17.6%) at locus LHMS14 and 4/459 (0.9%) at the other nine loci combined. In females these rates were 13/180 (7.2%) and 30/1620 (1.8%). The number

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Table 1 *L. hemichalceum* microsatellite loci. Number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E) and F_{IS} are for 180 female bees. P -values for tests whether F_{IS} is greater than zero are based on randomization tests permuting alleles within samples. * $P < 0.05$, *** $P < 0.001$ not corrected for the number of tests

Locus	Repeat motif	Primer Sequences 5'–3'	Annealing Temp. (°C)	GenBank Accession No	N_A	Allele Size Range	H_O	F_{IS}	H_E
LHMS1	(GA) ₄ GC(GA) ₆	F: ATCGCCTTATCAACTCAA R: TAGAGGGACGCAATCTGT	50	AF454674	19	94–136	0.774	0.037	0.804
LHMS2	(CT) ₁₄	F: GCTTCGGCTTTCTCTCTCT R: AACCCCGCTAAAATGACC	50	AF454675	9	148–164	0.798	–0.029	0.776
LHMS3	(CT) ₁₉	F: ACTCGCCGCAACATCCAG R: TTGAACCGGCACGAAAGAAC	58	AF454676	12	71–103	0.845	–0.055	0.801
LHMS4	(CT) ₁₈	F: TTCCTTTGTGAGCACTTA R: GACGGTGTTCGATGTTTC	50	AF454677	16	77–107	0.798	0.024	0.818
LHMS5	(CT) ₂₁	F: CGGTGGTTGGAAAACTA R: GGACGCTTCAGATTCAAG	50	AF454678	15	100–150	0.764	0.051	0.805
LHMS6	(CT) ₉	F: TCTGCCTGGAATAATACA R: GAAATGAGCTAGAGAGAACAA	50	AF454679	10	161–187	0.680	0.091*	0.748
LHMS10	(GA) ₁₂	F: GGGAGGGAGAACCAATG R: CAGCATCGCCAGAAAAAC	50	AF454680	14	92–134	0.818	0.011	0.827
LHMS14	(CT) ₁₆	F: GACGACGCTAACCCATG R: GACACCCGCTGCAAACAG	50	AF454681	22	93–139	0.646	0.231***	0.840
LHMS17	(GCGGC) ₄	F: GCGAGATTAGAAGGGTGG R: CGTGGACAGCGATTTTGG	50	AF454682	8	152–170	0.657	–0.040	0.632
LHMS18	(AC) ₁₄	F: CTTGTTTCCGCTCGTCTATA R: GAAGTCGTTACAGAACCCTGA	50	AF454683	15	114–142	0.785	0.029	0.808

of blanks in males was correlated with colony F_{IS} ($R^2 = 0.54$; $P < 0.05$) while the number of blanks in females ($R^2 = 0.15$) was not. Estimates of null allele frequency based on both F_{IS} and blank genotypes (Brookfield 1996; eqn 5) ranged from near zero in several colonies to as high as 0.43 in the smallest colony. To avoid bias relatedness values and incorrect exclusion of relationships (Pemberton *et al.* 1995) we omitted LHMS14 and used only nine loci for genetic analysis.

Detailed information on the genetic structure of *L. hemichalceum* social groups can now be obtained using these highly variable microsatellite loci. In addition to increasing understanding of communal sociality in *L. hemichalceum*, these microsatellite loci may be useful for closely related *Lasioglossum* species and will provide the opportunity to compare the social structure of communal *Lasioglossum* in Australia. Because *Lasioglossum* is a large, world-wide genus of Halictine bees it is particularly important in the study of social evolution. All levels of social structure from solitary to highly social are represented in this genus. Therefore, microsatellite loci that can be used for *Lasioglossum* are especially useful.

Acknowledgements

We thank F. Allendorf, J. Behm, K. Dahlberg, K. Hogendoorn, R. Leijss, S. Mills, and A. Parish. LHMS17 and 18 were sequenced by the University of Chicago Cancer Research Center. Supported by NSF awards DEB-973884 and DBI-0079265 to P.K.

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