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PRIMER NOTE

Characterization of microsatellite loci from the solitary sweat bees *Lasioglossum leucozonium* and *Lasioglossum oenotherae* (Hymenoptera, Halictidae)

AMRO ZAYED
Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

Abstract

Microsatellite loci were isolated from two solitary sweat bees: the polylectic *Lasioglossum leucozonium* (10 loci) and the oligolectic *Lasioglossum oenotherae* (9 loci) (Hymenoptera, Halictidae). All loci were polymorphic with high observed heterozygosities (0.07–0.75 for *L. leucozonium*; 0.06–0.92 for *L. oenotherae*). These loci will be used to study the consequences of diet specialization on the population and conservation genetics of bees.

Keywords: Apoidea, conservation genetics, diet specialization, Hymenoptera, microsatellite

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Bees provide a crucial ecosystem service through pollination, and this service is coming under increasing threats due to a seemingly worldwide decline in pollinator populations (Buchmann & Ascher 2005). A significant proportion of the world's bee fauna is oligolectic, where females collect pollen from one or a few closely related plant species (i.e. specialists), yet data on their population genetics are largely lacking (Packer *et al*. 2005; Zayed *et al*. 2005). Comparative data are needed to examine the possible consequences of specialization on the population genetics of oligolectic bees. I isolated microsatellite loci from the closely related *Lasioglossum* (*Sphecodogastra*) *oenotherae* (Stevens), oligolectic on Onagraceae, and the generalist *Lasioglossum* (*Lasioglossum*) *leucozonium* (Schrank) to facilitate population genetic studies of both species across North America.

I constructed a microsatellite-enriched library for both species using the methods outlined by Glenn & Schable (2005). Genomic DNA was extracted from the thoraces of two females per species using a DNeasy Tissue Kit (QIAGEN), followed by ethanol precipitation to concentrate the extracted DNA (~100 ng/µL). The genomic DNA (~10 µg) from each bee was then digested with either *Rsa*I or *Bst*UI (NEB) and ligated to SuperSNX24 linkers (forward, 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′; reverse, 5′-pGATTCTGCTAGCTAGGCCTTAAACAAAA-3′). Two mixes of 3′ biotinylated probes were used to enrich the libraries for the following microsatellite motifs: mix 1: $(AC)_{13}$ $(ATCC)_5$ $(AAGG)_5$ $(AAGC)_5$ $(AACG)_5$, and $(AACC)_5$; mix 2: $(TG)_{12}$ $(AG)_{12}$ $(AAG)_8$ $(ATC)_8$ $(AAC)_8$ $(AAT)_{12}$, and $(ACT)_{12}$. Linker-ligated DNA from each bee was hybridized to both probe mix 1 and 2 in two separate reactions following Glenn & Schable (2005). DNA fragments that hybridized with the probes were captured using streptavidin-coated magnetic beads (Dynal, Dynabeads M-280) and were later eluted by denaturing (Glenn & Schable 2005). The eluted DNA was used in a polymerase chain reaction (PCR) using the SuperSNX24 forward primer to recover the enriched DNA, which was subsequently cloned using a TOPO TA cloning kit (Invitrogen). Recombinant clones (179 for *L. leucozonium*, and 305 for *L. oenotherae*) were chosen from several enrichments and the insert in each clone was amplified using M13(–20) and reverse primers found in the vector (following Glenn & Schable 2005). PCR products were then visualized on an agarose gel alongside a MassRuler DNA ladder (Fermentas) to determine insert size and concentration.

Inserts between 500 and 1200 bp were cleaned using 5 U exonuclease I (NEB) and 0.75 U shrimp alkaline phosphatase (USB) by incubation at 37 °C for 15 min then 80 °C for 15 min, and the inserts were later sequenced using a CEQ DTCS Quick Start Kit on a CEQ 8000 (Beckman Coulter) with M13(–21) forward and reverse primers. Primers were developed to amplify some of the discovered microsatellite loci using PRIMER 3 (Rozen & Skaletsky 2000).

Correspondence: A. Zayed, Fax: 416-736-5698; E-mail: azayed@yorku.ca

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Table 1 Polymorphic microsatellite loci for *Lasioglossum leucozonium* (Lleu) and *Lasioglossum oenotherae* (Loen)

Locus	GenBank Accession no.	Primer sequence (5′–3′)	Sequenced repeat motif	T_a (°C)	n	N_A	Size (bp)*	H_O	H_E
Lleu-A12	DQ524973	F: CCCTAGAGAGTCGGTGAAAAGA R: TCCTTCCTTCTTTTGCTTGC	$(AGGA)_{10}$	60	16	2	184–188	0.25	0.23
Lleu-A13	DQ524974	F: CTGAAAAACGCTCGCAAAC R: ATGGATGGGAGAGTGAGACG	$(AC)_8(GCAC)_2CCACAT(AC)_5$	60	16	2	231–235	0.56	0.51
Lleu-A22	DQ524975	F: CCCCCTCTCTCACTCACTCA R: TCGTTGTTGAACCTCTCACG	$(CT)_7CC(CT)_8TT(CT)_3$ $TTCT(CA)_6TA(CA)_3$	60	16	2	193–225	0.50	0.51
Lleu-A52	DQ524976	F: ATTGCCAGAAGGGAGGAAAT R: GCCCTTATCTGTTTCCACGA	$(CT)_2(CCT)_2(CT)_{10}$	60	16	3	182–188	0.44	0.60
Lleu-A71	DQ524977	F: AAATTGTTCCGTCACTTTTGAA R: TATGCTCAGGCGCATCATAA	$(TG)_{12}$	60	15	2	208–210	0.07	0.07
Lleu-A73	DQ524978	F: AGGATACTGATTGCCGTGCT R: CGAAGAAGCATCACTTCACA	$(GT)_2CT(GT)_{10}$	60	13	3	223–233	0.38	0.60
Lleu-B27	DQ524979	F: CATTCGCCATCACCATTACC R: AGCAAAGACGAGAGGGAACA	$(TCT)_2TCC(TCT)_2TCC(TCT)_2TCA(TCT)_3$ $(TCC)_2(TCT)_4 \ldots (CGT)_2(CCT)_2(CGT)_{10}$	60	16	2	217–220	0.50	0.44
Lleu-B34	DQ524980	F: GCTGCGGAATAAAACAGAGG R: GTCGTCATTCCTATGCGTCA	$(GA)_3GGG(GA)_2AA(GA)_{10}$	60	15	2	177–179	0.27	0.24
Lleu-B60	DQ524981	F: TCAGCTCCAACCCCATACAT R: CGAACAAAGGAGAGGGAGAG	$(GA)_{18}GG(GA)_3$	60	16	3	254–276	0.75	0.57
Lleu-B72	DQ524982	F: CACGAAGGTGAAACGACTGA R: CGGGATACACGGCTATCTTC	$(GA)_6CA(GA)_3AA(GA)_2CA(GA)_6$	60	16	3	216–232	0.50	0.63
Loen-A31	DQ524983	F: AGAAGGATGAGCCACGAATG R: ATTTTCCCGGACACTGGTTT	$(GA)_{13}GT(GA)_8$	60	13	7	224–236	0.92	0.81
Loen-A33	DQ524984	F: ACCGGACATTTACTTATCCTCG R: TTCATGTATCTAGCTCTACGGGAA	$(TG)_6C(GT)_{17}$	60	16	8	291–341	0.75	0.75
Loen-C8	DQ524985	F: ACGTTCGCCGGAAAATCTATTG R: CAAACTGCATGGGCGTGG	$(TC)_{21}$	60	16	10	225–281	0.81	0.85
Loen-D5	DQ524986	F: TTCTCTCTCGCCTTCACTCC R: TTCATCTTTCTCGGGTTTCG	$(CT)_7 \ldots (CGT)_8$	60	16	5	189–201	0.75	0.70
Loen-D53	DQ524987	F: CGCGTCTTTCCAGTTCAAA R: ACACGACTTTTCCGTCGTTC	$(AG)_{10}TG(AG)_{11}AA(AG)_4$	60	13	13	288–336	0.85	0.89
Loen-D68	DQ524988	F: GTGCATCCCCATTGTCAAC R: GGTATCCGTTTTCCCTCGTC	$(CT)_6(C)_5(CT)_8TTAT(CT)_3TCTT(CT)_2$	60	13	3	193–197	0.46	0.45
Loen-D71	DQ524989	F: GCCTCGTCTTCAAAGGAATG R: TGGTTACTCTGGACCGATGA	$(TC)_3C(TC)_{15}TT(TC)_5$	60	16	8	188–226	0.69	0.83
Loen-E6	DQ524990	F: TTAGTCCTCGCTCGACCAGT R: CTCAAGGGAGCATCGAAAAA	$(TCT)_7(CCT)_2$	60	14	2	183–189	0.21	0.20
Loen-E10	DQ524991	F: CACCAGGTTGTTGTTCTCCA R: CTGCTACCTTCGTCCAGCTC	$(GA)_2(TTG)_7(ATG)_3$	60	16	2	217–223	0.06	0.06

F, forward primer; R, reverse primer; T_a, annealing temperature; n, diploid sample size; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. The genotyped females were sampled from Albion Hill, Ontario, Canada (43°56.05′N, 79°49.92′W) and Ithaca, New York, USA (42°27.12′N, 76°29.78′W) for *L. leucozonium* and *L. oenotherae*, respectively.
*Fragments amplified and fluorescently labelled using the universal M13 protocol (see methods) will be 19 bp larger than the reported values.

I examined the variability of the discovered loci in a sample of approximately 16 females per species (Table 1) using a nested 10 µL PCR reaction (following Schuelke 2000) containing 20–40 ng DNA, 0.3 µM reverse primer, 0.075 µM forward primer with a 5′ M13 tail (5′-TGTAAAACGACGGCCAGT + forward primer), 0.3 µM M13(–21) primer labelled with either D2, D3 or D4 WellRed dyes (IDT), 1× *Taq* Buffer with KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, and 0.2 U *Taq* DNA polymerase (all Fermentas). The reactions were performed in a Mastercycler gradient thermocycler (Eppendorf) with the following conditions: 94 °C for 5 min; 30 cycles at 94 °C for 30 s, at the primer-specific annealing temperature (Table 1) for 45 s, 72 °C for 45 s; 8 cycles at 94 °C for 30 s, 53 °C for 45 s, 72 °C for 45 s, and a final extension at 72 °C for 10 min. Fragment analysis was subsequently performed on a CEQ 8000 (Beckman Coulter), by pooling two PCR products (1 µL each) labelled with different dyes with 36 µL de-ionized formamide (Fermentas) and 0.5 µL 400 bp size standard (Beckman Coulter).

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All loci were polymorphic with high observed and expected heterozygosities; however, *L. oenotherae* exhibited higher allelic richness than *L. leucozonium* (Table 1). None of the loci exhibited significant departures from Hardy–Weinberg equilibrium ($P > 0.05$), as indicated by randomization tests in FSTAT version 2.9.3 (Goudet 1995). FSTAT was also used to test for linkage disequilibrium between pairs of loci for each species. For *L. leucozonium*, out of 45 pairwise comparisons, linkage disequilibrium was detected between *Lleu-B34* and *Lleu-B60* ($P = 0.04$), but this association did not remain significant when corrected for multiplicity of tests. Linkage disequilibrium was not detected among any pair of *L. oenotherae* loci. The hypervariability of the characterized loci will greatly facilitate population genetic studies within *Lasioglossum* and may be useful for related bees.

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