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Development and characterization of eleven microsatellite markers for a popular pet stag beetle, *Dorcus hopei* (Coleoptera, Lucanidae) using paired-end Illumina shotgun sequencing

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Abstract

Eleven polymorphic microsatellite loci were developed and characterized for *Dorcus hopei* in this study. The number of alleles varied from 2 to 21. The observed heterozygosity and expected heterozygosity ranged from 0.1058 to 0.9744 and 0.0997 to 0.8941, respectively. Two loci showed low polymorphism, while the rest were highly polymorphic. Six loci deviated from Hardy-Weinberg Equilibrium. The set of markers will provide effective tools for examining the population genetic structures and be helpful for managing wild population in *D. hopei*.

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Introduction

A stag beetle, *Dorcus hopei* belonging to the family Lucanidae is a famous insect pet and widely reared by many insect fanciers between Korea and Japan. In Japan, to increase the body size the fanciers had crossbred the stag beetles from Japanese and Taiwanese populations (Miwa, 2011) and then the same fashion has been concerned in Korea. These artificial rearing of the species may cause serious and potential risks in ecosystems. Furthermore, the Korean wild population has been declined (Park *et al*., 2001), but genetic status of its populations is still unknown. To facilitate a better understanding of genetic diversity and population structure of *D. hopei* for future conservation plan, we performed development of microsatellite markers of *D. hopei* using Illumina paired-end genomic sequencing.

Materials and Methods

Genomic DNA (gDNA) was extracted from thorax muscle tissue of a wild male (voucher no. 8421, collecting date: 9th April 2013) using a DNeasy Blood and Tissue kit (QIAGEN). An Illumina paired-end shotgun library was prepared by sharing 200 ng of gDNA using a Covaris S220 and following the standard protocol of the Illumina TruSeq nano DNA Library Kit and using a multiplex identifier adaptor index. Selection for microsatellite markers, Genotyping and Statistical Analyses were according to Castoe *et al*. (2012) and Silva *et al*. (2013).

Results

A total of 30,050,470 reads were generated. The total number

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Table 1. Characterization of 11 microsatellite loci in *Dorcus hopei*

Locus	primer sequence (5'→3')	Repeat motifs	Tm (℃)	Size Range (bp)	GenBank accession No.	*N*	*K*	*Ho*	*HE*	*PHW*	PIC
1084	FAM-ACCTAGTTCCGTATTATTTG	(AATT)4	51	242-246	KJ740763	76	2	0.1058	0.0997	1.0000	0.0948
	TACGATATTTATCTCGGAGA		51								
112495	FAM-CGAAATAGAGTGAAGAGATT	(AAAT)4	51	263-271	KJ740766	76	3	0.1184	0.1358	0.0113	0.1295
	TTTCAATCCAAGAAAGGTTAC		52								
14434	FAM-TCTTCCATCCTATATGACAT	(CGT)5	51	255-291	KJ740769	77	9	0.5195	0.7434	0.0000*	0.7068
	TATCCTTGCTGTTTTATTCAAT		50								
36256	FAM-TCAAAAACTTTTTCTCCATG	(GT)6	49	235-275	KJ740787	77	8	0.5455	0.7641	0.0000*	0.7243
	GAGCAGGGAGATAAATAAAA		51								
102941	FAM-CTACGTATGGTATCACTTTTGCTTT	(AAT)24	57	191-344	KM270757	77	10	0.5195	0.5869	0.0773	0.5557
	CAGCTTTTTGTATTTTCGTAATGTAC		55								
2720	FAM-AAATACGTATTTCGATGTCTAAGTTG	(CTT)20	55	106-250	KM270758	77	21	0.8312	0.8941	0.0000*	0.8861
	GAAGGCTTTCAATTTAACATTACTAA		53								
16781	FAM-AACAGAAAATACCGCATATATGTAGC	(AGT)19	57	122-224	KM270759	78	19	0.9744	0.8292	0.0060	0.8113
	TTTTTCACACAAACAATTCATATCGAC		55								
101378	FAM-ATACTGCACTGTTCTATTTTTTCAGA	(AG)24	55	249-344	KM270760	75	10	0.3333	0.6388	0.0000*	0.6077
	CCATACATATTTTGTCGAAATTTATGAG		55								
41149	FAM-AGTGTATTGTTGTGTGATAAATTTTG	(GT)22	53	135-155	KM270761	76	12	0.8553	0.6763	0.0007*	0.6565
	TGAAGTTTTCCGTTTTATTCTAAGA		53								
95146	FAM-GTGTTAGAAAAGGAAAGTTCTGGATC	(CT)21	58	207-245	KM270762	73	10	0.4247	0.7217	0.0000*	0.693
	AAGCTATTTTCTACGTTTCTCAGTTCG		58								
84678	FAM-GGTTTTCGTATAGTTGTCTATCTGT	(GT)20	57	169-349	KM270763	77	10	0.8052	0.8261	0.0273	0.8032
	ACAATCTTCATTTGCATTTTCTGGTG		57								

N number of individuals genotyped, *K* number of alleles observed, *Ho* observed heterozygosity, H_E expected heterozygosity, *PIC* polymorphism information content, ($P_{HW} < 0.0045$) * Significant deviation

of contigs was 123,299 and the length of average contigs was 2,710 bp. Tri-nucleotide repeats were the most abundant class of microsatellites (33,436 regions) detected in the partially assembled *D. hopei* genome, followed by di-nucleotide (2,993 regions), tri-nucleotide (12,367 regions) and tetra-nucleotide (1,623 regions). The most frequent types of microsatellite sequences on each class were detected as AC, ATT and ATTT, respectively. The most frequent tri-nucleotide type in the *D. hopei* genome was ATT repeats (20.3%), followed by AAT (20.2%), CTT (6.1%) and AAG (6%).

Sixty microsatellites were selected with the sequentially largest number of repeat motifs to test amplification efficiency and assess polymorphism. We assessed the variability of the 11 polymorphic loci in 78 specimens. The 11 new microsatellite markers were deposited in the GeneBank database under accession numbers KJ740763–KM270763 (Table 1). These loci showed successfully amplified PCR products. Conditions and characteristics of the loci are provided in Table 1. The number of alleles ranged from 2 to 21, the expected heterozygosity and observed heterozygosity values ranged from 0.0997 to 0.8941 and 0.1058 to 0.9744, respectively. Two loci (1084 and 112495) showed low polymorphism ($0 < PIC < 0.25$), while the rest were highly polymorphic ($PIC > 0.5$). Tests for deviations from Hardy-Weinberg equilibrium (P_{HW}) and linkage disequilibrium were conducted that six loci deviated from P_{HW} after Bonferroni corrections. Linkage disequilibrium was detected for three of the 55 paired loci (14434 and 95146; 102941 and 84678; 95146 and 84678) comparisons. These microsatellite markers will be useful for accessing population genetic structures and helpful for managing declining wild population in *D. hopei*.

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