information that will be useful for conservation and restoration efforts for this endangered species.

Acknowledgements

The authors wish to thank Rungtiwa Punyayod, Kulthida Pakkad and Kulnaree Pakkad for helping to collect plant materials. This study was financially supported by Grant-in-Aid from the Japan Society for the Promotion of Science (JSPS).

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doi: 10.1111/j.1755-0998.2008.02334.x

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Characterization of 10 microsatellite loci in an avian louse, *Degeeriella regalis* (Phthiraptera: Ischnocera: Philopteridae)

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Abstract

We isolated and characterized 10 polymorphic microsatellite loci in an ischnoceran louse, *Degeeriella regalis*, which parasitizes the threatened Galápagos hawk (*Buteo galapagoensis*) and other falconiform birds. The loci were screened across 30 individuals from two island populations in the Galápagos Islands. The number of alleles per locus ranged from two to 28. Polymorphic information content ranged from 0.14 to 0.94 and observed heterozygosity ranged from 0 to 0.67. These markers will be valuable in comparative population genetics studies in this species, which is the focus of a long-term population and disease ecology research program.

Keywords: avian louse, *Degeeriella regalis*, ectoparasite, Galápagos hawk, microsatellites, population genetics

Received 23 May 2008; revision accepted 16 July 2008

Comprising *c*. 3100 species, the ischnoceran lice are an extremely diverse component of the parasite fauna infesting birds and mammals (Price *et al*. 2003). Ischnoceran lice typically harbour significant genetic diversity within

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populations and high structure among populations (Whiteman *et al*. 2007 and references therein), but many previous studies relied on allozyme or mitochondrial DNA sequence data. Although microsatellite loci have served as powerful molecular fine-scale markers in other parasite species, including human lice (Phthiraptera: Anoplura) (Leo *et al*. 2005), there are no reports of microsatellite loci from ischnoceran lice.

Interactions between five ectoparasite species (including *D. regalis*) and the Galápagos hawk (*Buteo galapagoensis*), have been the subject of intense study in the Galápagos Islands (Whiteman *et al*. 2007 and references therein). Population structure and history of *D. regalis* tracked that of the host across the species range in the Galápagos Islands; however, fine-scale molecular markers are required to study its transmission dynamics in greater detail.

For initial microsatellite development, DNA was extracted using QIAGEN DNeasy kits from *D. regalis* lice originally collected from *Buteo galapagoensis* individuals live-captured on two islands in the Galápagos (Isabela and Santiago), following Whiteman *et al*. (2007). For the initial enrichment and microsatellite isolation, DNA was extracted from ca. 100 poded louse individuals collected from a single host. Only a single louse was extracted from a host individual for the variability assessment. DNA was serially enriched twice for microsatellites using three probe mixes (2, 3, and 4) following Glenn & Schable (2005). Briefly, DNA was digested with restriction enzyme *Rsa*I (New England Biolabs) and simultaneously ligated to double-stranded SuperSNX linkers. Linker-ligated DNA was denatured and hybridized to biotinylated microsatellite oligonucleotide mixes, which were then captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was washed away and remaining DNA was eluted from the beads, amplified in polymerase chain reactions (PCR) using the forward SuperSNX as a primer, and cloned with TOPO TA Cloning Kits (Invitrogen). We isolated inserts from a total of 187 clones using bacterial colony PCR with M13 forward and reverse primers and sequenced using the same primers and BigDye version 3.1 (Applied Biosystems) on an ABI-3130xl sequencer. Sequences from both strands were assembled and edited in Sequencer 4.5 (Gene Codes). Microsatellites were identified and primers designed using MSATCOMMANDER (Faircloth 2008). One primer in each pair was modified on the 5′ end with an engineered sequence (CAG tag; see http://www.uga.edu/srel/DNA_Laboratory/protocols.htm).

We tested 56 primer pairs for amplification and polymorphism on eight lice from the Isabela population. PCR amplifications were performed in 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/mL BSA, 0.4 μM unlabelled primer, 0.08 μM tag-labelled primer, 0.36 μM universal dye-labelled primer, 1.2–3.4 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 U JumpStart *Taq* DNA Polymerase (Sigma), and 2–6 ng DNA). Touchdown PCR programmes spanned 10 °C of annealing temperatures between 65 and 55 °C, 60–50 °C or 55–45 °C (see Table 1). Cycles were 95 °C for 3 min; five cycles of 95 °C for 30 s, highest annealing temperature for 30 s, and 72 °C for 30 s; 21 cycles of 96 °C for 30 s, highest annealing temperature (decreased 0.5 °C per cycle) for 30 s, and 72 °C for 30 s; and 15 cycles of 96 °C for 30 s, lowest annealing temperature for 30 s, and 72 °C for 30 s. Loci Dre211 and 224 were cycled at a single annealing temperature of 62 °C for 47 cycles and 66 °C for 35 cycles, respectively. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard prepared as described in DeWoody *et al*. (2004), except that unlabelled primers started with GTTT. Dre217 was sized with GeneScan-500 ROX size standard. Results were analysed using GeneMapper version 4.0 (Applied Biosystems). Ten of the tested primer pairs showed polymorphism across eight individuals. DNA sequences for each of these 10 loci have been deposited in GenBank (Table 1).

We assessed variability of these 10 loci on a total of 30 lice from two populations: Isabela (n = 15) and Santiago (n = 15). PCR conditions and characteristics of the 10 loci are given in Table 1. We used Cervus version 3.0 (Kalinowski *et al*. 2007) to estimate number of alleles per locus (k), observed and expected heterozygosities, and polymorphic information content. Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were determined using GenePop version 4.0 (Rousset 2008). Two loci, Dre23 and Dre224, deviated from expectations of HWE in the Isabela population after Bonferroni correction for multiple comparisons. No linkage was detected among 90 paired loci comparisons in two populations after Bonferroni correction. We used Micro-Checker version 2.2.3 (Van Oosterhout *et al*. 2004) to detect the presence of null alleles which may explain observed departures from HWE. No loci showed significant evidence of null alleles.

This set of variable loci will allow for fine-scale population genetic studies in *D. regalis* in the Galápagos Islands. Because *D. regalis* has also been reported from at least nine other bird species (Price *et al*. 2003), these microsatellite markers (the first developed for an ischnoceran louse) are likely to be relevant outside of the context of the Galápagos Islands.

Acknowledgements

This work was supported in part by the US Department of Energy, through Financial Assistance Award no. DE-FC09–07SR22506 to the University of Georgia Research Foundation, by a Research Board grant from the University of Missouri System, by the National Science Foundation (NSF; INT-030759), the National Institutes of Allergy and Infectious Diseases (F32AI069732), the Field Research for Conservation Program (FRC) of the Saint Louis Zoo, Harris World Ecology (UM-St Louis), Sigma Xi, and the E. Desmond Lee Collaborative in Zoological Studies. TAME provided discounted roundtrip air-travel. For the Galápagos sampling and permits, we thank the Servicio Parquè Nacional de Galápagos and the Charles Darwin Research Station. Tjitte de Vries and students

17550998, 2009, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2008.02363.x by Univ of California Lawrence Berkeley National Lab, Wiley Online Library on [03/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1 Characterization of 10 polymorphic microsatellite loci genotyped in 30 individuals of *Degeeriella regalis* from two populations in the Galápagos Islands: Isabela (Isa) and Santiago (San). T_a corresponds to highest annealing temperature for touchdown thermal cycling; $MgCl_2$ is an optimized concentration for magnesium chloride; size indicates the range of observed alleles in bp; N is the number genotyped for each population; k is number of alleles observed in each population; H_O and H_E are observed and expected heterozygosities of each population, respectively, and PIC is polymorphic information content of each locus.

Locus/GenBank Accession no.	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	$MgCl_2$ (mm)	Size (bp)	N (Isa/San)	k (Isa/San)	H_O (Isa/San)	H_E (Isa/San)	PIC
Dre12/EU768861	F: [CAG].GAGCAAGACACAAGTGCCC	$(AG)_7$ ... $(AG)_6$	55	2.0	181–183	15	2	0.467	0.434	0.255
	R: GTTTCGTTCGGGGTAAGTGCTC					15	2	0.133	0.129	
Dre13/EU768862	F: GTTTGGTTTCTTCTGGGGTTACACG	$(A)_{13}$ ... $(ATC)_8$	55	2.0	407–413	15	2	0.333	0.287	0.141
	R: [CAG].AGCAAACAGCGGAAGACAG					15	1	0	0	
Dre23/EU768864	F: GTTTCGTAGAAACCTGCCTGCG	$(GAT)_{15}$	65	2.0	175–202	15	5	0.467*	0.761	0.582
	R: [CAG].ACACTCTTCATTTTCCAAAACAGG					15	3	0.4	0.393	
Dre202/EU768859	F: GTTTCGGGAGAAGGAGACGGAG	$(AG)_9$	65	2.0	232–236	15	2	0.4	0.331	0.193
	R: [CAG].TCCGGGCAAAGAAATTGAAC					15	2	0.067	0.067	
Dre204/EU768860	F: [CAG].ACGAATAGGGAAAACGGGG	$(ATC)_9$	65	2.0	184–190	15	3	0.533	0.432	0.478
	R: GTTTAGCGGAAGACAGAAGAGCG					15	2	0.533	0.497	
Dre206/EU768868	F: GTTTCCCTTCACTCACTCACTCCC	$(AC)_{36}$	60	2.0	235–257	15	4	0.4	0.356	0.421
	R: [CAG].GCCACCACCTGTGATAAACG					15	4	0.467	0.552	
Dre211/EU768863	F: [CAG].CTTCGAGTCCTTCCCTCCC	$(CT)_{30}$	62†	3.4	204–226	13	7	0.462	0.708	0.721
	R: GTTTCGTCGCTCTAAGCCAAAGTG					12	5	0.667	0.703	
Dre217/EU768865	F: [CAG].GCAGTCAGAAACACCCTGC	$(ATGT)_{63}$	60	2.0	409–461	15	3	0.133	0.248	0.180
	R: GTTTGAACCTCCTCCCGTACCAG					15	3	0.133	0.131	
Dre223/EU768866	F: CTTTTACAGTGGTTTCATTCTTGTC	$(GT)_{10}$	55	2.0	215–219	15	2	0.267	0.515	0.476
	R: [CAG].AAACAAGACCATTTCTCGGC					15	3	0.333	0.508	
Dre224/EU768867	F: GTTTACCCCGATGATAATACGTTGC	$(CT)_{27}$	66†	1.2	185–291	15	15	0.667*	0.931	0.942
	R: [CAG].ATACCCGCTCCATTTCCCC					15	17	0.8	0.943	

*significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction.
†annealing temperature for loci for which touchdown PCR was not used. Primers with superscript [CAG] have been modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′) and fluorescently labelled with FAM.

(Pontificia Universidad Catolica del Ecuador) and Jennifer Bollmer (UM-St Louis) provided help with fieldwork and advice.

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doi: 10.1111/j.1755-0998.2008.02363.x

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