Eight polymorphic microsatellite markers isolated from the widespread avian louse *Colpocephalum turbinatum* (Phthiraptera: Amblycera: Menoponidae)

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Abstract

We report eight novel microsatellite loci for *Colpocephalum turbinatum*, a parasitic louse of the endangered Galápagos hawk (*Buteo galapagoensis*). Two island populations of *C. turbinatum* (*N* = 30) were genotyped for each locus. We found between two and 12 alleles per locus, polymorphic information content from 0.268 to 0.798, observed heterozygosity from 0.067 to 0.667 and no linkage disequilibrium was detected between loci. These markers will be useful in understanding contemporary gene flow of *C. turbinatum* among islands in the Galápagos and in understanding transmission dynamics between *B. galapagoensis* hosts, within and between social groups. Because this louse is unusually widespread among avian host taxa, parasitizing at least 53 bird species in the Falconiformes, Strigiformes and Columbiformes, these markers are likely to be useful outside the context of the Galápagos Islands.

Keywords: avian louse, ectoparasite, Galápagos hawk, population genetics

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Comprising *c*. 1300 described species, the amblyceran lice comprise a large proportion of the ectoparasite fauna infesting birds and mammals (Price *et al*. 2003). Although microsatellite loci have served as powerful molecular fine-scale markers in other parasite species, including human lice (Phthiraptera: Anoplura) (Leo *et al*. 2005), and markers have been developed for an ischnoceran louse (*Degeeriella regalis*; Peters *et al*. in press), there are no reports of microsatellite loci from amblyceran lice. Allozyme studies on amblyceran lice (*Heterodoxus* spp.) in Australia showed low variation within and high differentiation among colonies of rock wallaby hosts (Barker *et al*. 1991), similar to the findings of Whiteman *et al*. (2007), which focused on mitochondrial variation in the avian louse *Colpocephalum turbinatum*, among island populations of the Galápagos hawk (*Buteo galapagoensis*). In contrast, Goméz-Díaz *et al*. (2007) found little variation within and structure among populations of *Austromenopon echinatum* from *Calonectris* shearwaters. The development of fine-scale molecular markers could yield important new insight into the population dynamics of amblyceran lice.

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Interactions between the ectoparasite community (including *C. turbinatum*) and the Galápagos hawk (*B. galapagoensis*) have been the subject of intense study in the Galápagos Islands (Whiteman *et al*. 2007 and references therein). Fine-scale molecular markers are required to study its transmission dynamics in greater detail, particularly in the context of a long-term study on the influence of host social structure on the genetic structure of *C. turbinatum* within and among host social groups.

We collected *C. turbinatum* lice from separate host *B. galapagoensis* individuals on two Galápagos islands (Fernandina and Santiago) following Whiteman *et al*. (2007). For the initial microsatellite enrichment and isolation, DNA was extracted from 100 pooled lice (from a single *B. galapagoensis* host) using QIAGEN DNeasy kits. This DNA sampled was serially enriched twice for microsatellites using probe mixes 1, 2 and 3 described in Glenn & Schable (2005). DNA was digested with *Rsa*I (New England Biolabs) and ligated to SuperSNX linkers (F 5′-GTTTAAGGCCTAGCTAGCAGC-AGAATC; R 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA).

Table 1 Characteristics and conditions of eight polymorphic microsatellite loci isolated from *Colpocephalum turbinatum*

Locus/GenBank Accession no.	Primer sequence 5′→3′	Repeat motif	T_a (°C)	Size (bp)	N (Fer) (San)	k (Fer) (San)	H_O (Fer) (San)	H_E (Fer) (San)	PIC
Ctu06/EU915459	F: ^{CAG}TCTCTGTCTCTCACTCTCATTCTC	$(CT)_6$ ... $(CT)_7$ ...	55	377–420	15	2	0.133	0.129	0.332
	R: GTTTCCCCACAATTACACCAACCG	$(CT)_7$			15	2	0.4	0.515	
Ctu08/EU915460	F: GTTTAAACCTTTGTAACGGCCATC	$(AC)_7$ ... $(AT)_7$	55	268–270	15	2	0.267	0.331	0.305
	R: ^{CAG}CTTTACTGACCCGAGCAACG				15	2	0.333	0.434	
Ctu25/EU915461	F: GTTTCCGGCGAACTTTGCTCTTG	$(AG)_9$	55	257–259	15	2	0.133	0.239	0.305
	R: ^{CAG}ATTTCTGCAACAGCGCCC				15	2	0.333	0.480	
Ctu26/EU915462	F: ^{CAG}GCAACTAATTTGTGTCCGTGC	$(CT)_7$ ... $(T)_{10}$	55	284–289	15	2	0.067	0.067	0.268
	R: GTTTCACGTGGGAGTCAAACC				15	3	0.4	0.480	
Ctu29/EU915463	F: GTTTGGCGTTTGACGCTCTTTTC	$(CT)_{25}$	55	225–264	15	3	0.267	0.301	0.767
	R: ^{CAG}GTGGCATAGATAAGCTGATGGG				15	10	0.667	0.899	
Ctu35/EU915464	F: ^{CAG}GCTCGCACACTGGTAACAC	$(AC)_{11}$	55	208–219	15	6	0.467	0.729	0.798
	R: GTTTGTGCAAAGTCAACAAGCGG				15	7	0.667	0.821	
Ctu207/EU915465	F: GTTTCCCGGCGCACAAGTTAATG	$(CTT)_8$	65†	251–257	15	3	0.333	0.600	0.348
	R: ^{CAG}TTGGGTAGAACCCTGCCAC				15	2	0.067	0.067	
Ctu235/EU915466	F: GTTTCTGGTCGCCGCTGTTTTAG	$(AG)_{11}$	60	255–260	15	6	0.333*	0.775	0.689
	R: ^{CAG}AATCGTGATGCCTCTCAGC				14	5	0.429	0.704	

Loci were screened in two populations, Fernandina (Fer) and Santiago (San), in the Galápagos Islands. T_a corresponds to highest annealing temperature in a touchdown thermal cycling programme; size indicates the range of observed alleles in base pairs; 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. *indicates significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction, and †indicated a reduced number of cycles in a touchdown thermal cycling programme. Primers with superscript CAG have been modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′) and fluorescently labelled with NED.

Linker-ligated DNA was denatured, hybridized to biotinylated oligonucleotide mixes, and captured on magnetic streptavidin beads (Dynal). Unhybridized DNA was removed, and remaining DNA eluted, polymerase chain reaction (PCR) amplified using the forward SuperSNX24 as a primer, and cloned with TOPO TA Cloning Kits (Invitrogen). Inserts from a total of 186 clones were amplified directly from the bacterial colonies via PCR (Applied Biosystems 9700) and sequenced with M13 forward and reverse primers using BigDye Terminators version 3.1 (Applied Biosystems) and an ABI 3130xl sequencer. Sequences were assembled in Sequencher 4.1 (Genecodes) and microsatellites identified using Msatcommander version 0.8.1 (Faircloth 2007). One primer from each pair was modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′) to enable use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labelled (ABI) for detection (see Schable *et al*. 2002).

To screen these loci for variability, DNA was extracted from individual lice (each from a different *B. galapagoensis* host) using QIAGEN DNeasy kits. Ninety primer pairs were initially screened on eight lice. PCR was performed in a 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, 2 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 U JumpStart *Taq* DNA polymerase (Sigma)], and 2–6 ng DNA template (on an Applied Biosystems 9700 thermocycler). Touchdown PCR (Don *et al*. 1991) spanned 10 °C of annealing temperatures between 65–55 °C, 60–50 °C or 55–45 °C (Table 1). Cycles were 95 °C for 3 min; 5 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 20 s, highest annealing temperature (decreased 0.5 °C per cycle) for 20 s, and 72 °C for 30 s; and 15 cycles of 96 °C for 20 s, lowest annealing temperature for 20 s, and 72 °C for 30 s. Ctu207 was cycled at a touchdown programme with 10 cycles at lowest annealing temperature. PCR products were run on an ABI 3130xl sequencer and sized with Naurox size standard ranging from 82–428 bp with a total of 12 fragments (DeWoody *et al*. 2004; unlabelled primers started with GTTT). Results were analysed using GeneMapper version 4.0 (Applied Biosystems).

A total of 126 of the inserts from the 186 clones contained repeats. Eight of the 90 tested primer sets amplified polymorphic PCR products. We further assessed these loci on a total of 30 lice from two populations (each from a different host individual): Fernandina (n = 15) and Santiago (n = 15).

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We determined the number of alleles per locus, observed and expected heterozygosities, polymorphic information content, and frequency of null alleles using Cervus version 3.0.3 (Kalinowski *et al*. 2007). No linkage was detected among 56 paired loci comparisons in two populations after Bonferroni correction. Deviations from Hardy–Weinberg equilibrium and linkage disequilibrium were determined using GenePop version 4.0 (Raymond & Rousset 1995). Ctu235 showed significant deviations from Hardy–Weinberg equilibrium in the Fernandina population after Bonferroni correction ($P = 0.0043$). We employed the Brookfield method (Brookfield 1996) implemented in Micro-Checker version 2.2.3 (van Oosterhout *et al*. 2004) to detect the presence of null alleles. All loci had estimated null allele frequencies < 0.27.

This set of variable loci will allow for fine-scale population genetic studies in *C. turbinatum* in the Galápagos Islands and elsewhere. Because this species has also been reported from at least 52 other bird species from three orders (Price *et al*. 2003), these microsatellite markers (the first developed for an amblyceran louse) are likely to be useful in many other contexts. In particular, *C. turbinatum* may comprise a species complex exhibiting cryptic host specificity among host lineages (Price & Beer 1963). Evidence in support of this is given by the strong population genetic structure present in *C. turbinatum* on island populations that have diverged recently (Whiteman *et al*. 2007).

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