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Microsatellite primers from *Geospiza fortis* and cross-species amplification in Darwin's finches

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Studies of Darwin's finches have addressed the heritibility of morphological traits (Grant & Grant 1994), effective population size (Grant & Grant 1992), hybridization (Grant & Grant 1994) and adaptive radiation (Lack 1947; Grant 1986). Conclusions based on these investigations depend on correct identification of genetic relationships. Microsatellites have the unique potential to resolve questions of genetic relationships at all of these levels (McDonald & Potts 1997).

Sixteen polymorphic microsatellite loci were characterized in a species of Darwin's finch: *Geospiza fortis*, the medium ground finch. Primers for these loci amplify polymorphic products in a wide range of Darwin's finch species, as well as some more distantly related buntings and sparrows. I present data on variation across species to serve as a guide for their general utility.

Blood was collected by brachial vein puncture and dried in the presence of 0.5 M EDTA (Petren *et al*. 1999). Total DNA was extracted using a combination of salt precipitation (Miller *et al*. 1988) and phenol extraction (Sambrook *et al*. 1989). Alternatively, a guanidine-based method (QIAGEN, Ausubel *et al*. 1988) was used for rapid processing. A genomic library was constructed and screened generally following Primmer *et al*. (1995). Total DNA was digested with *Sau*3AI, and 200–500 bp fragments were purified by agarose gel electroelution (Sambrook *et al*. 1989), and ligated into a CIP-treated pUC plasmid. Competent cells (SURE cells, Stratagene) were transformed by electroporation and plated at high density on six large plates (Nunc, 23 × 23 cm). More than 1.2×10^6 colonies were screened by colony lift and hybridization with an equimolar mixture of six [^{32}P]-ATP end-labelled di- and trimeric motif oligonucleotides (Ausubel *et al*. 1988; Primmer *et al*. 1995). A total of 150 colonies with the strongest signal was picked, rescreened, and 70 colonies were miniprepped and cycle sequenced (fmol kit, Promega). Cells were kept on ice during the screening procedures. Primer pairs (N = 26) were picked by eye with preference given to clones with > 13 uninterrupted repeats. Of these, 21 yielded single amplification products, with 16 showing polymorphisms.

After optimization, genotyping was carried out in a 96-well format (both total DNA dilutions and PCR reactions) using a multichannel pipette, which greatly facilitated processing. PCR reactions (10 μL) contained 25 ng of DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each primer (12.5% of one primer end-labelled with [^{32}P]-ATP) and 0.5 units of *Taq* polymerase (Promega). Thermocycling conditions were 94 °C for 40 s, 50 °C for 40 s, 72 °C for 40 s, repeated 33 times. Some loci required higher annealing temperatures (55 °C: Gf09, Gf13; 62 °C: Gf08, Gf11, Gf15), and some required lower annealing temperatures (44 °C) and reduced extension times (20 s, Gf02, Gf03). Products were electrophoresed in 6% acrylamide gels (90/gel) with cycle-sequenced pUC plasmid size standards, and three gels were exposed to a single sheet of film to facilitate scoring (by eye).

The 16 polymorphic *Geospiza fortis* loci (Table 1) are also highly polymorphic in related genera (Table 2). Eight of these loci were able to detect extrapair paternity with > 99% certainty in a different species (*G. scandens*, Petren *et al*. 1999). This contrasts with previous findings in birds that found a steep decline in polymorphism at most loci even among closely related genera (Primmer *et al*. 1996), and may reflect the close relationships among Darwin's finches.

Polymorphism at all 16 loci is relatively high among most species of Darwin's finches, suggesting that they may be valuable for studies of hybridization and phylogenetic inference. One study of over 700 individual birds from 13 species of Darwin's finches has shown that phylogenetic signal is present and generally concordant among these loci (K. Petren, B. R. Grant, P. R. Grant, unpublished). Studies of hybridization and population variation are currently in progress.

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Table 1 Characteristics of 16 microsatellite loci developed in *Geospiza fortis*. (N = 36 birds genotyped). H_O and H_E is the observed and expected heterozygosity, respectively. Nucleotide sequences for cloned fragments have GenBank accession numbers AF081925–AF081940

Locus	Core repeat	No. of alleles	Size range	H_O	H_E	Primer sequence (5'–3')
Gf01	$(AC)_{23}$	6	159–191	0.79	0.80	F: TAGCATTTCTATGTAGTGTTATTTTAA R: TTTATTTATGTTCATATAAACTGCATG
Gf02*	$(AC)_{11}$	8	123–141	0.51	0.81	F: AGACTATACTTCTAGCTTATATTTATC R: ATACATATAAAATGGTAAGGTGTATA
Gf03	$(GA)_{17}$	9	184–204	0.89	0.81	F: CCAGCTTAAAGCCAGCACTTCC R: CAAAGTTTTATTCTGAATGAATAAAG
Gf04	$(AC)_{11}$	3	186–200	0.60	0.56	F: CCTTTGCAAAACCGGGTCTG R: TTTTCTTATATCTATTGAGAGATGGT
Gf05	$(AC)_{14}$	5	200–218	0.36	0.35	F: AAACACTGGGAGTGAAGTCT R: AACTATTCTGTGATCCTGTTACAC
Gf06	$(AC)_{13,6}$	3	175–179	0.25	0.52	F: GCTATTGAGCTAACTAAATAAACAACT R: CACAAATAGTAATTAAAAGGAAGTACC
Gf07	$(AC)_{22}$	8	186–220	0.78	0.73	F: TTCTGGAATTGCTTGTAAATAG R: GCACTATCTCACAGTGATATCTAAAAT
Gf08	$(AC)_{21}$	9	101–139	0.80	0.81	F: TGGGAGAGCAAGGTGGGAACAG R: TGGAGTGGTGATTAAACCAGCAGG
Gf09	$(AC)_{14}$	8	143–169	0.89	0.82	F: AGTCAAGAACAGGACACTAACAACT R: TGGAAGGACTCCAGAGATGCTGT
Gf10	$(AG)_{17}$	5	216–226	0.35	0.55	F: TTGAGGGTCCCATCCAACTG R: GGTGCTCTTGATACAAAGCATAA
Gf11	$(AC)_{28}$	11	168–224	1.00	0.88	F: GTGCTATCAGCGAGGCATTTC R: AGGAGGATTTGGCTGACTGG
Gf12	$(AC)_{17}$	11	167–193	0.83	0.82	F: AATCCTTCTCGTCCCTCTTGG R: TTTGAGTGTGCAGCAGTTGG
Gf13	$(AC)_{14}$	10	149–177	0.61	0.75	F: TCCCCCGTGAAAAGTGGAGC R: CAACACAATTGCAATATCGATTCCC
Gf14	$(AC)_{20}$	5	125–141	0.56	0.46	F: TTTTTACAGAGCTTCTACAATTATAGC R: TCAAAAAATTGCATTAATTCTG
Gf15	$(AC)_{14}$	4	184–202	0.52	0.46	F: CTCCACCTCCCACTAACTGCTACC R: CAACACCTGGAGTGGAAGTGCC
Gf16	$(AC)_{15}$	9	126–148	0.74	0.80	F: CCCTTCAGGGCATGAGTGAGG R: ATGTCATGAACTCAACCAACTCC

*Alleles at this locus are inherited in a sex-linked (Z-linked) manner.

Table 2 The number of alleles detected in other species with microsatellite markers developed in *Geospiza fortis*

Species	(N)	Gf01	Gf02*	Gf03	Gf04	Gf05	Gf06	Gf07	Gf08	Gf09	Gf10	Gf11	Gf12	Gf13	Gf14	Gf15	Gf16
Geospiza scandens	(68)	8	4	7	2	7	4	7	8	4	6	11	8	6	6	2	9
Geospiza conirostris	(49)	11	3	7	4	5	4	10	11	3	3	11	6	8	8	4	7
Camarhynchus parvulus	(22)	13	7	7	2	5	1	6	15	5	1	7	9	7	7	4	3
Certhidea olivacea	(20)	12	6	2	3	3	8	5	14	14	1	5	12	7	4	2	2
Tiaris olivacea	(17)	7	3	0	3	5	11	1	2	2	2	1	6	8	7	1	2
Sporophila aurita	(8)	5	2	0	2	7	3	1	2	1	4	1	7	2	2	1	1
Zonotrichia albicollis	(3)	5	0	0	1	2	2	2	0	0	1	2	3	1	3	2	2

*Alleles at this locus are inherited in a sex-linked (Z-linked) manner.

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Isolation and characterization of microsatellite markers in the bloodsucking bug *Rhodnius pallescens* (Heteroptera, Reduviidae)

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Chagas' disease or American trypanosomiasis is caused by *Trypanosoma cruzi*, which is transmitted to humans in the faeces of some bloodsucking bugs that commonly live in human dwellings (Schofield & Dolling 1993). Disease outbreaks may result from the combined effects of destruction of wild habitats of the bugs, and building of suitable alternative habitats, i.e. houses. The interruption of the epidemiological cycle relies mainly upon vector control (WHO 1997). However, reinfestation of human dwellings previously sprayed with insecticide has been frequently observed probably due to a persistence of residual insect foci, incidental human transport of bugs, or active invasion of houses by bugs from neighbouring infested houses or wild habitats such as palm trees for *Rhodnius* spp. One method of testing the recolonization hypothesis is to analyse the genetic structure of wild and domestic bug populations and estimate the dispersal abilities and hence gene flow of the vectors. However, such investigations require appropriate genetic markers. Previously available isoenzyme markers, first revealed in *Triatoma infestans* (Dujardin & Tibayrenc 1985) are not polymorphic enough for fine-scale studies. For example, in *Rhodnius prolixus* populations the average heterozygosity is less than 0.05 for 19–10 loci (Harry *et al.* 1993; Lopez & Moreno 1995). Therefore, we turned to microsatellites which are highly polymorphic genetic markers that have recently been shown to be suitable for population genetic studies of two *Anopheles* vector species (Lanzaro *et al.* 1995; Rongnoparut *et al.* 1996).

A partial genomic library was constructed from genomic DNA of *R. pallescens* digested with *Sau*3A (Boehringer Mannheim) according to Estoup *et al.* (1993). Restriction fragments were cloned in pUC18 vector (Pharmacia). The screening procedure was performed on 4000 clones with a mix of six DIG-labelled oligonucleotide probes: $(TG)_{10}$, $(CT)_{10}$, $(CAC)_{10}$, $(CCT)_{10}$, $(ATCT)_5$ and $(TGTA)_6$. Of the 93 positive clones isolated (*c.* 2.3% of clones have a microsatellite sequence), 44 were sequenced using the T7 sequencing kit (Pharmacia). The primers were designed by using OLIGO software (Rychlik & Rhoads 1989).

Ten microsatellites were selected (Table 1) to test the variability of a *R. pallescens* population sampled from naturally occurring palm trees *Attalea butyraceae* (Arecaceae) in Colombia (San Onofre, Sucre). We amplified these loci from one bug collected from each of 36 trees. PCR amplifications were performed using a Biometra thermal cycler, with 30 cycles of: 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s. The reaction (10 μL) contained 1× PCR buffer (50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl, pH 9.0), 300 μM of each dNTP, 0.2 μg/μL BSA, 1.2 mM $MgCl_2$, 0.0092 μM forward primer end-labelled with $[\gamma^{32}P]$-dATP, 0.0128 μM unlabelled forward primer, 0.4 μM unlabelled reverse primer, 0.25 units of *Taq* polymerase (Boehringer), and approximately 10 ng of template DNA. For L17, the reaction mixture was modified as follows: 1 mM $MgCl_2$, 75 μM dCTP, DGTP, dTTP, 6 μM dATP, 0.4 μM of each primer, 25μCi/μL $[\alpha^{33}P]$-dATP.

For the 10 microsatellites studied, the number of alleles per locus ranged from two to 20 and the expected heterozygosity ranged from 0.32 to 0.94 (Table 1). The proportion of heterozygotes was as expected at Hardy–Weinberg equilibrum for all the markers except L13, L17, L47 and L58 (GENEPOP, Raymond & Rousset 1995). We examined the ability of primers to amplify appropriately sized products in other bug species. No PCR products were obtained in *Triatoma infestans* but successful amplification occurred in two other *Rhodnius* species, namely *R. ecuadoriensis* (L3, L9, L13, L25, L43, L47) and *R. prolixus* (L43, L47).

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