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MICROSATELLITE LETTERS

Autosomal and Z-linked microsatellite markers enhanced for cross-species utility and assessed in a range of birds, including species of conservation concern

Deborah A. Dawson[1] · Susannah Bird[1,2] · Gavin J. Horsburgh[1] · Alexander D. Ball[1]

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Abstract Microsatellite markers were designed to be of utility for genotyping multiple species of birds, including those of conservation concern, hence saving resources and enabling species/genome comparisons. We used the proven approach of Dawson et al. (Mol Ecol Resour 10:475–494, 2010) and assessed markers in multiple species, including nine species of conservation interest. We ensured both primer sequences matched multiple species (13 loci) or designed primer sets from expressed sequence tags (2 loci). Eleven primer sets were 100 % identical to the zebra finch (*Taeniopygia guttata*) and a second passerine species and/or the chicken (*Gallus gallus*). All 15 loci were polymorphic when assessed in a non-source species (Gouldian finch, *Erythrura gouldiae*) suggesting utility in multiple species. Four of the five Z-linked loci were assessed in at least nine additional species each (including ratites). All were variable in multiple species, demonstrating cross-species utility and potential for identifying Z chromosome rearrangements.

Keywords AVES · Birds · High cross-species utility · Passerine · Simple tandem repeat (STR) · Z chromosome

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✉ Deborah A. Dawson
d.a.dawson@sheffield.ac.uk

1 Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

2 Present Address: Department of Biology, University of York, York YO10 5DD, UK

Introduction

In order to create enhanced microsatellite markers of high cross-species utility we followed the approach of Dawson et al. (2010). We designed primer sets from alignments of multiple species/expressed sequence tags and characterised these in non-source species. Those markers identified as Z-linked based on sequence homology to the zebra finch and genotyping were tested in additional bird species. Z markers with wide cross-species amplification are particularly useful because species-specific allele sizes allow the identification of the parental species of a hybrid individual (e.g. Lifjeld et al. 2010), whereas the more variable Z markers allow the study of cross-species chromosomal rearrangements (e.g. Backström et al. 2006).

Methods

We followed the approach of Dawson et al. (2010) to enhance the markers for high cross-species utility. We created consensus zebra finch–passerine, zebra finch–chicken or zebra finch–passerine–chicken sequences from homologous microsatellite sequences using MEGA3 (for details see Supplementary File 1). Primer sets were designed from these multi-species consensus sequences using PRIMER3 v0.4.0 using a maximum of one degenerate base per primer set.

All markers were assessed in a non-source species, the near-threatened Gouldian finch (*Erythrura gouldiae*). Since this species was not used in the design of the markers, successful amplification and polymorphism would suggest the marker will be of utility in many species. We tested known sexes to diagnose if the markers were Z-linked or autosomal (Table 1; up to 20 female and 20 male; sexed

Table 1 Fifteen microsatellite markers of enhanced cross-species utility and characterised in estrildid finches

Primer set name	Repeat motif	Chr	Primer sequences and fluoro-label (5′–3′)	Sp tested	N	Observed allele size range (bp)	A	H_o	H_e	Species with 100 % match to both primers	Passerine sequence aligned (Locus, GenBank/EMBL accession no. and reference)
O142-ZFC	GT	1	F: [6-FAM]-TCTCTTGCCTGAAGGCTCTC	GF	6	234–236	2	0.17	0.17	ZF, P	O142, AY696193
			R: CATCTGCTTCwCCAAGACATTC								Burgess and Fleischer (2006)
Ase52-ZEST	CA	3	F: [HEX]-TCTAACACATCTGAAAACCAGCTAC	GF	18	210–215	5	0.89	0.78	ZF, P (EST)	Ase52, AJ276781
			R: TTTTCTTGATGCATATTATGGTGTC								Richardson et al. (2000)
Ase60-ZFS	GT	3	F: [HEX]-GGCTTGCTTTTATTGTATCATGC	GF	9	174–184	3	0.22	0.60	ZF, P	Ase60, AJ276789
			R: CAGGACTGGCATATTAGAAATGTTTAC								Richardson et al. (2000)
ApCo104-ZFC	CAA	5	F: [HEX]-TCTGCTGACGACTTTATTACCC	GF	8	117–123	3	0.25	0.54	ZF, P	ApCo104, AF520900
			R: TTTCCCTCTCgTAACACTGC								Stenzler and Fitzpatrick (2002)
Ase24-ZFS	GA	5	F: [HEX]-TGTGCATGTGTGTGCATTTG	GF	14	201–215	5	0.79	0.62	ZF, P	Ase24, AJ276381
			R: TGTTGTCTGAAGCTGTCATTGC								Richardson et al. (2000)
Ase12-ZFS	CA	7	F: [6-FAM]-TCATCCATCAAGGAAACACAAC	GF	18	120–136	8	0.39	0.84	ZF, P	Ase12, AJ287395
			R: TCCTCACAGCCTTGACTGG								Richardson et al. (2000)
Cdi31-ZFM	CT	7	F: [6-FAM]-GAACTTCTGCATTTGTTCCTCTC	GF	6	139–141	2	0.50	0.41	ZF, P	Cdi31, AB089172
			R: GAGAGCGTGCTGAATGAGTG								Otsuka et al. (2003)
DkiD12-ZF (Chr 9)	GATA	9	F: [6-FAM]-GCTTGGCAATTAAAAACTCAA	GF	8	181–191	2	0.38	0.53	ZF, P	DkiD12, AY769684
			R: CAAGACACTGAGGCATCAAA								King et al. (2005)
ZEST09-005	CA	9	F: [6-FAM]-AACCCAACCAACAAAATTGG	GF	7	154–158	3	0.43	0.39	ZF (EST)	ZEST09-005, DV954446
			R: CCAACTATCAGTTTTACAAGGCATAC								Replogle et al. (2008)[a]
ZEST09-018	AT	9	F: [6-FAM]-TGTCTTGTATTTGCTACCATATCACTG	GF	14	283–293	7	0.71	0.84	ZF (EST)	ZEST09-018, CK307510
			R: ATCCTGCAGTGCTGCTTCTC								Replogle et al. (2008)[a]
Ase46-ZFM	GT	Z	F: [6-FAM]-CTGGCTGTATCTTGGTGTGC	GF	2M	143–147	2	0	0	ZF, P	Ase46, AJ276775
			R: GCTAACTTTCCATTGAACTGTCC	GF	3F	143–147	3	0	0		Richardson et al. (2000)
Z-013	AT	Z	F: [6-FAM]-GGTAGmTTTTTAAAGCCCAGAT	GF	6M	296–302	4	0.33	0.64	ZF, CH	Z-013, CK311793
			R: TTGACTGTACAAATACAGCAAGTT	GF	9F	296–298	3	0	0		Replogle et al. (2008)
				ZF	10M	294	1	0	0		
				ZF	9F	294	1	0	0		
Z-037	AT	Z	F: [6-FAM]-AAAACACCTTGTAATTTAAAACTGG	GF	7M	164–168	3	0.71	0.71	ZF, CH[b]	Z-037, DV945670
			R: CATAGATACATATCAATACAGCACATTC	GF	11F	164–168	3	0	0		Replogle et al. (2008)
				ZF	20M	170, 172	2	0.25	0.22		
				ZF	20F	170, 172	2	0	0		
Z-040	AT	Z	F: [6-FAM]-AAAAGTCTTTCTGGACTGTGCT	GF	6M	122–136	7	0.83	0.88	ZF, CH	Z-040, DV949035
			R: AAAATACAACAGACATAGGCATACA	GF	9F	124–130	4	0	0		Replogle et al. (2008)

Table 1 continued

Primer set name	Repeat motif	Chr	Primer sequences and fluoro-label (5′–3′)	Sp tested	N	Observed allele size range (bp)	A	H_o	H_e	Species with 100 % match to both primers	Passerine sequence aligned (Locus, GenBank/EMBL accession no. and reference)
				ZF	20M	171, 173	2	0.15	0.14		
				ZF	20F	171, 173	2	0	0		
Z-054	CA	Z	F: [6-FAM]-CTGTCTGGCATGCTGACTC	GF	6M	279–296	7	0.83	0.86	ZF, P, CH	Ase50, AJ276779
(Ase50-Gga)			R: ATCAGCAGACACAACATGGACTC	GF	9F	288–296	8	0	0		Richardson et al. (2000)
				ZF	10M	262–293	6	0.70	0.82		
				ZF	9F	264–293	4	0	0		

Eleven characterised in the endangered Gouldian finch (*E. gouldiae*) and four Z-linked markers characterised in the Gouldian finch and the zebra finch (*Taeniopygia guttata*). Chr, predicted chromosome in zebra finch (*T. guttata*); degenerate base IUB codes: W = A or T, M = A or C; *N* number of individuals genotyped, *A* number of alleles observed, H_o observed heterozygosity, H_e expected heterozygosity, *GF* Gouldian finch, *ZF* zebra finch, *P* passerine (non-ZF), *CH* chicken, *EST* expressed sequence tag, *M* male, *F* female; [a] see also Ball et al. (2010); base mismatching bases in zebra finch are shown underlined (ApCo104-ZFC and Ase12-ZFS), [b] for *Z-037* the first 5′ base "A" of the forward primer mismatches in chicken

using the marker Z-002A, Dawson 2007). Four of the Z markers were genotyped in 10–23 additional species (including nine species of conservation concern) and the saltwater crocodile *Crocodylus porosus* (Table 2; 1–40 individuals per species).

Genomic DNA was extracted from blood using an ammonium acetate protocol. Each fluorescent PCR contained approximately 10 ng genomic DNA, in 2-µl volumes using QIAGEN Multiplex PCR Master Mix or 10-µl volumes with 2.5 mM $MgCl_2$ and BIOLINE *Taq* DNA polymerase and buffer. PCR amplification was performed using a DNA Engine Tetrad thermal cycler. PCR amplification conditions were 94 °C for 15 min (QIAGEN) or 3 min (Bioline); then 35 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; followed by one cycle of 72 °C for 10 min. PCR products were loaded on a 48-capillary ABI 3730 DNA Analyzer and genotypes assigned using GeneMapper software (Applied Biosystems). Heterozygosities were calculated using cervus and deviation from Hardy–Weinberg equilibrium (HWE) calculated using genepop. Z-linked markers were assessed for HWE when typed in a minimum of 10 individuals and using males only.

Results

Eleven primer sets were 100 % identical to zebra finch and a second bird species and two sets mismatched at 1–4 bases (ApCo104-ZFC and Ase12-ZFS; Supplementary File 1). Two other sets were designed from zebra finch Expressed Sequence Tags (ESTs) (ZEST09-005 and ZEST09-018; Table 1).

Based on their high multispecies sequence homology and proven utility in multiple non-source species (Table 1, 2), these markers are expected to be of utility for studying many species, including those of conservation concern, especially passerines (see Dawson et al. 2010). Only one locus (*Ase12*) deviated from HWE, possibly due to null alleles (Table 1). A selection of these markers are being used in parentage studies for multiple species (DAD unpublished data).

For the five loci homologous to the zebra finch Z chromosome, females were always homozygous (hemizygous), confirming their Z-linked nature in other species, and suggesting no W-linked homologues amplified (Table 1). Four of the Z-linked sequences were highly conserved between genetically distant species (zebra finch-chicken; *Z-013*, *Z-037*, *Z-040* and *Ase50*) and, as expected, amplified across a wide range of species, including ratites and saltwater crocodile (81–100 %; Table 2, Supplementary Table 1). These were variable in multiple species demonstrating potential for cross-utility and identifying

Table 2 Four Z-linked bird markers enhanced for cross-species utility and assessed in a wide range of bird species (including nine of conservation concern) and the saltwater crocodile (*Crocodylus porosus*)

Order, family	Species *binominal name*	Species	IUCN (2014) status[a]	Ase50 (Z-054)	Z-013	Z-037	Z-040	Sample supplier
REPTILE	*Crocodylus porosus*	Saltwater crocodile	(LC)	–	268–280	178	147	Winston Kay
BIRDS								
RATITES								
Palaeognathae, Struthioniformes	*Struthio camelus*	Ostrich	LC	–	265, 364	159, 163	125	Jeff Graves
Palaeognathae; Apterygiformes	*Apteryx australis*	Brown kiwi	Vulnerable	–	245–256	–	–	Maori Leaders Council & Department of Conservation, New Zealand
Palaeognathae; Casuariiformes	*Dromaius novaehollandiae*	Common emu	LC	–	262 (423)	–	–	Dominique Blache
Palaeognathae; Casuariiformes	*Casuarius casuarius*	Southern cassowary	Vulnerable	–	262–278	–	–	Leon Huynen
Palaeognathae; Rheiformes	*Rhea pennata (Pterocnemia)*	Lesser rhea (Darwin's rhea)	LC	–	259–278	–	–	Andy Balmford
NON-RATITES								
Anseriformes	*Anas platyrhynchos*	Mallard	LC	–	–	159	127	Emma Cunningham
Anseriformes	*Cairina moschata*	Muscovy duck	LC	–	261	–	–	Moshen Vaez
Columbiformes	*Streptopelia picturata rostrata*	Seychelles turtle dove	Unknown	–	260	–	–	David Richardson
Columbiformes	*Streptopelia turtur*	Turtle dove	LC	–	–	160	125	Olivier Hanotte
Coraciiformes	*Merops apiaster*	European bee-eater	LC	–	269	–	–	Kate Lessells
Coraciiformes (Bucerotiformes)	*Tockus monteiri*	Monteiro's hornbill	LC	–	262	159	127	David Richardson
Cuculiformes	*Cuculus canorus*	Common cuckoo	LC	–	–	159	124	Bengt Hansson
Falconiformes	*Aquila chrysaetos*	Golden eagle	LC	–	265	161	127	Brian Bourke
Falconiformes	*Falco cherrug*	Saker falcon	Endangered	239	–	160	PCR fail	Andy Dixon
Galliformes	*Alectura lathami*	Brush turkey	LC	–	256	–	–	Darryl Jones
Galliformes	*Gallus gallus*	Red junglefowl	LC	243, 251	256, 258	159	119	Hans Cheng
Gruiformes	*Grus paradisea*	Blue crane	Vulnerable	–	263	159	PCR fail	Kate Meares
Passeriformes	*Pica pica*	Black-billed magpie	LC	274	–	–	–	David Martín-Gálvez
Passeriformes	*Turdus merula*	Eurasian blackbird	LC	283, 289	–	161	No amp.	Ben Hatchwell
Passeriformes	*Cercomacra tyrannina*	Dusky antbird	LC	–	268, 271	–	–	Terry Burke
Passeriformes	*Erythrura gouldiae*	Gouldian finch	Near Threatened	279–296	295–302	162–168	122–136	Simon Griffith and Sarah Pryke
Passeriformes	*Acrocephalus arundinaceus*	Great reed warbler	LC	–	–	158	179–181	Bengt Hansson
Passeriformes	*Parus major*	Great tit	LC	260	–	163	138	Harrie Bickle

Table 2 continued

Order, family	Species *binominal name*	Species	IUCN (2014) status[a]	Ase50 (Z-054)	Z-013	Z-037	Z-040	Sample supplier
Passeriformes	*Passer domesticus*	House sparrow	LC	–	–	166	135	Nancy Okendon
Passeriformes	*Corvus monedula*	Eurasian jackdaw	LC	268–270	–	–	–	Ian Henderson
Passeriformes	*Aegithalos caudatus*	Long tailed tit	LC	–	–	161	127	Ben Hatchwell
Passeriformes	*Emberiza schoeniclus*	Reed bunting	LC	–	–	167	PCR fail	Graeme Buchanan
Passeriformes	*Zosterops lateralis chlorocephala*	Silver eye	Unknown	–	–	166	135	Ian Owens
Passeriformes	*Sturnus vulgaris*	Starling	LC	–	–	165	125	Mike Double
Passeriformes	*Pycnonotus xanthopygos*	White-spectacled bulbul	LC	–	–	167	142	Jon Wetton
Passeriformes	*Taeniopygia guttata*	Zebra finch	LC	262–293	294	169–172	171, 173	Tim Birkhead
Piciformes	*Melanerpes formicivorus*	Acorn woodpecker	LC	–	258–264	–	–	Joey Haydock
Procellariiformes	*Macronectes giganteus*	Southern giant petrel	LC	–	–	159	127	Richard Phillips
Psittaciformes	*Strigops habroptilus*	Kakapo	Critically Endangered	–	260	–	–	Bruce Robertson
Psittaciformes	*Nestor notabilis*	Kea	Vulnerable	–	–	159	125	Terry Burke
Sphenisciformes	*Pygoscelis adeliae*	Adelie penguin	Near Threatened	–	–	159	127	Fiona Hunter
Sphenisciformes	*Eudyptes chrysolophus (pachyrhnchus)*	Macaroni penguin	Vulnerable	–	259	–	–	Richard Phillips
Strigiformes	*Tyto alba guttata*	Barn owl	LC	–	259	–	–	Akos Klein
Strigiformes	*Athene noctua*	Little owl	LC	278	–	–	–	Akos Klein
Strigiformes	*Asio otus*	Northern long-eared owl	LC	278	–	–	–	Akos Klein

[a] IUCN (2014) classification (extracted from http://www.iucnredlist.org/search): *LC* least concern, *No amp.* no amplification, *PCR fail* failure to amplification probably due to PCR problems, – not tested; Summary data for the four Z chromosome microsatellite markers is provided in Supplementary Table 1

chromosomal rearrangements between species. Allele sizes in some orders were invariant within a species, yet varied in size between species, suggesting potential for identifying species and hybrids (Table 2).

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