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Microsatellite markers for the Cape Robin Chat (*Cossypha caffra*) and the Red-Capped Robin Chat (*Cossypha natalensis*) for use in demographic and landscape genetics analyses

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Abstract: The Robin-chats (Muscicapidae: *Cossypha*) are distributed across sub-saharan Africa with many species restricted to small fragments of Afromontane forest. Several species have decreasing population trends, so demographic data and landscape genetic data for these species will be essential for conservation management. Here we develop 23 microsatellite markers for two species of *Cossypha* (*C. caffra* and *C. natalensis*), characterize polymorphism, and cross-amplify a subset of loci. We demonstrate that most markers have high information content with many alleles suggesting that these markers will be useful for assessing population dynamics and demography. Several loci cross-amplified between species and retained high polymorphism, indicating that these loci will likely be of high utility for many species of African Robins.

The Robin-chats of the genus *Cossypha* primarily occur among the montane sky islands of Africa forming an important component of the Afromontane forest community assemblage. Several species have highly restricted distributions, and populations of several species appear to be in decline; one Robin-chat species (*C. heinrichi*) is presently listed as Vulnerable by IUCN. In order to evaluate demographic trends and patterns of gene flow, we first develop microsatellite markers *for C. caffra* and *C. natalensis*, and then cross-amplify the loci to assess their utility across the genus.

Microsatellite enrichments followed the protocol of Glenn and Schable (2005). For *Cossypha caffra* we used RsaI and XmnI cutters, while for *C. natalensis* we performed two digestions using RSA. SuperSNX24 linkers were ligated onto the DNA fragments and enriched using biotin labeled tetranucleotide probes: $(AAAT)_8$; $(AACT)_8$; $(AAGT)_8$; $(ACAT)_8$; $(AGAT)_8$. For *C. natalensis* we also used trinucleotide probes: $(ACT)_8$; $(ACG)_8$; $(AAG)_8$; $(ATC)_8$; $(AAC)_8$. For *C. caffra* a total of 83 colonies were sequenced, 57 of which contained repetitive elements. For *C. natalensis* 234 colonies were sequenced, 162 of which contained repetitive elements.

Enriched fragments with 6-8 tetra or penta -nucleotide repeats were selected for additional development. Primers were designed for 19 *C. caffra* loci and 16 *C. natalensis* using WebSat (Martins et al. 2009) and Primer3 (Rozen and Skaletsky 2000), respectively. The forward primers were 5' tagged with a flourophore (Table 1).

Genotypic data were generated from a Malawi population (n=14) of *C. natalensis,* and a South African population (n=12) of *C. caffra.* Loci were amplified and genotyped using the same PCR conditions as in Wogan *et al.* (in review). To evaluate the potential utility of these loci among Robin-chats, we cross-amplified several loci.

We assessed the presence of null alleles, and calculated the number of alleles, size range of alleles, polymorphism information content (PIC), observed and expected heterozygosity (with 1000 repetitions), and then determined if any loci exhibited departures from Hardy-Weinberg Equilibrium (HWE) using the exact test. Analyses were performed in R and Microchecker (van Oosterhout et al. 2004) following Wogan *et al.* (in review).

For *C. natalensis*, we amplified 17 loci consistently, 13 species-specific and four developed from *C. caffra*. Each contained between 4 and 12 alleles, and the PIC was high, suggesting that the loci have high information content. Observed heterozygosity values ranged from 0.286 to 1.0. Several species-specific loci departed from HWE: CNA130, CNA137, CNA139, CNA233. Evidence for the presence of null alleles was observed for three loci: CNA137, CACA27 and CACA34.

For *Cossypha caffra*, 14 loci amplified consistently, ten species-specific loci and four developed from *C. natalensis*. Each contained from 3 to 8 alleles, and the PIC value was high for most loci. Observed heterozygosity values ranged from 0.167 to 1.0. Significant departures from HWE were detected for two species-specific loci: CACA55 and CACA66. There was no evidence for stuttering or large allele dropout in any of the loci; however, null alleles may be present for CNA233.

We found that many loci cross-amplified and had high variability, suggesting that these loci will be of potential use in other species of Robin-chats. We were able to amplify high quality data for 23 microsatellite markers across the two Robin-chats examined here. With additional optimization, more loci may successfully cross-amplify. These markers will be of high utility for estimating population genetic and demographic parameters for African robins (e.g. *Cossypha*, *Sheppardia*).

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Wogan GOU, Feldheim K, Voelker G, Bowie RCK (in review) Development and characterization of thirteen microsatellite markers for the Fiscal Flycatcher (*Sigelus silens*) for use in phylogeographic and landscape genetics research Conservation Genetics Resources

Table

Click here to download Table: Cossypha_PrimerNoteTable.doc

Locus	Genbank Accession	Primer Sequence (5'-3'), fluorescent label	Repeat motif	Ta (°C)	Size Range	N_A	H_E	H_O	PIC	HWE Exact	N_{AX}	H_{EX}	H_{OX}	PICx	HWE_x
CNA69	KM273236	F-NED-CCACCTTTAATACATTTCTAGTCAGTC R-TTGTCCTTCCAAAACCAACC	$(TGGA)_{13}$	54	152-198	6	0.707	0.857	.735	0.752	-	-	-	-	-
CNA99	KM273237	F-NED- GGGTTCCTGTTCCCTTCTCT R- CCATGTCCTGTGCATCTCAA	$(TGGA)_{11}$	54	106-137	7	0.793	1.0	0.818	0.781	-	-	-	-	-
CNA109	KM273238	F-HEX-GCACATATTGCCTTACAGTG R-AATTGCACAGGCTAATATG	$(GATG)_{14}$	52	170-214	9	0.752	0.786	0.770	0.701	-	-	-	-	-
CNA111	KM273239	F-FAM- CTAGCTAGCAGGCTCATTCG R-ATATGAGGCATGCAAGCCTG	$(TCCA)_{10}$	56	167-203	10	0.796	0.923	0.830	0.873	-	-	-	-	-
CNA113	KM273240	F-FAM- CAGCACTCAGGCAAATGAAA R-AGCAGCTCAGAAGGCAAAAC	$(TGGA)_{14}$	56	108-152	12	0.882	0.846	0.870	0.326	-	-	-	-	-
CNA130	KM273241	F-FAM-GTGATTAGCAGAGTTAGCTTC R-TCCACAGAAATCTCGAACAG	$(TGGA)_{10}$	54	147-184	9	0.804	0.643	0.779	0.008*	-	-	-	-	-
CNA137	KM273242	F-FAM- GGGATTGTCTTCTGCACTCAG R-CCTCAGTTTGATCCGTCCAC	$(TGGA)_{8}$	56	154-182	6	0.75	0.286	0.761	0.000*	-	-	-	-	-
CNA139	KM273243	F-FAM-CCTCAGTTTGATCCGTCCAC R-GACTCTAATCAAGATGAGAC	$(TCCA)_{13}$	56	325-338	4	0.618	0.429	0.601	0.000*	4	0.66	0.417	0.628	.004*
CNA142	KM273244	F-HEX-AAGCAAGGCAGGATGCTCAC R-TTGTCTATGATTCTTAGCAC	$(TGGA)_{13}$	54	181-213	8	0.843	0.923	0.831	0.948	2	.375	0.417	0.689	0.048
CNA162	KM273245	F-FAM-TGAAACTAAAAACACCAAGGAAA R- GCAATTTGTGAGCGCAACTA	$(ATGG)_{10}$	56	240-260	6	0.687	0.692	0.732	0.019	3	.632	0.167	0.764	0.000*
CNA180	KM273246	F-NED-ACATCTGCAGAGCACCATTG R-GAGCCAGGGAAGGAAGGAT	$(ATAC)_{9}$	56	101-125	5	0.714	0.857	0.733	0.111	-	-	-	-	-
CNA214	KM273247	F-NED-TATGCAGGACGTGCTTCCTAC R-TCTCTGAACACCAGTAGTAG	$(TCCA)_{11}$	56	227-259	7	0.781	1.0	0.829	0.505	-	-	-	-	-
CNA233	KM273248	F-HEX-TTGCCATTGAATTGGGAGTT R- GAGAGTCACCTGGGATGGAG	$(GATG)_{18}$	56	84-136	11	0.781	0.643	0.828	0.001*	4	.625	0.5	0.757	0.000*
CACA3	KM273249	F-HEX-GCTTGGAGGACTAACCAATGA R-AACTATTCCCTGCCTTTCTGTG	$(AGAT)_{11}$	60	312-320	3	0.559	0.583	0.559	0.103	8	0.799	0.714	0.777	0.056
CACA12	KM273250	F-HEX-GGACAGACAACACTTCATTTGG R-GCTGTGAGATTCAGGTTTGAGA	$(ATGG)_{12}$	56	378-398	6	0.708	.583	0.769	0.012	-	-	-	-	-
CACA26	KM273251	F-HEX-GCAGAATCACTGCTAATGACTGTT R-TCAAAACCAGACAAAGACTACAGG	$(AGAC)_{5}..(AGAT)_{9}$ $..(ATCC)_{10}$	54	317-361	6	0.673	0.667	0.795	0.014	-	-	-	-	-
CACA27	KM273252	F-HEX-TGTGTCCACGAGTAAAATCAGC R-AATACCCAGAAAACAAACTGGC	$(TAGA)_{12}$	61	336-348	4	0.656	0.75	0.725	0.071	9	0.85	0.214	0.864	0.000*
CACA34	KM273253	F-HEX-ATATTGAGGAAGGGAGAGAGGG	$(TGGA)_{13}$	60	115-135	6	0.785	0.818	0.843	0.037	8	0.805	0.231	0.847	0.000*

		R-TAGCAGAATCCACCAAGTTTGA													
CACA43	KM273254	F-FAM-TGTTAGGATAGGGGAAAGCGTA R- CTGTGCAAGCAGATGTAACTCC	$(AGAT)_{14}$	60	287-331	6	0.722	0.75	0.822	0.575	-	-	-	-	-
CACA55	KM273255	F-HEX-GGGCAGGTTAGAATGAGACAAC R-TCGATCAGTGGACAATCAAGTC	$(ATAG)_{13}$	62	303-407	8	0.708	0.75	0.781	0.002*	7	0.806	0.714	0.745	0.392
CACA56	KM273256	F-FAM-AACTAAAGGCCACAAGAATCCC R-TGCAGTCTCTCTCTCTTTCCATC	$(TATC)_{10}(ATCT)_{10}$	61	190-250	7	0.83	1.0	.878	0.900	-	-	-	-	-
CACA66	KM273257	F-HEX-AGGACTCACTTGTCATTCAGCA R-CATTCCATCCACTGTAAGCTCC	(ATGTT)9	58	302-332	6	0.652	0.583	.671	0.000*	-	-	-	-	-
CACA78	KM273258	F-HEX-GCTGTGCAAAATCCAAACAGTA R-GCAGAATCACACCAAGTATCCA	$(ATCC)_8ATC(ATCC)$ $ATC(ATCC)_8$	61	190-250	7	0.83	1.0	.878	0.928	-	-	-	-	-

Table 1. Characterization of microsatellite loci isolated from either *Cossypha natalensis* (CNA) or *Cossypha caffra* (CACA). Population measures for cross-amplified loci are demarcated with an x.