

UC Berkeley

UC Berkeley Previously Published Works

Title

Development and characterization of thirteen microsatellite markers for the Fiscal Flycatcher (*Sigelus silens*) for use in phylogeographic and landscape genetics research

Permalink

<https://escholarship.org/uc/item/9qw2496c>

Journal

Conservation Genetics Resources, 7(1)

ISSN

1877-7252

Authors

Wogan, Guinevere OU
Feldheim, Kevin A
Voelker, Gary
[et al.](#)

Publication Date

2015-03-01

DOI

10.1007/s12686-014-0309-2

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-NoDerivatives License, available at <https://creativecommons.org/licenses/by-nc-nd/4.0/>

Peer reviewed

Development and characterization of thirteen microsatellite markers for the Fiscal Flycatcher (*Sigelus silens*) for use in phylogeographic and landscape genetics research

Guinevere O. U. Wogan¹, Kevin A. Feldheim², Gary Voelker³, and Rauri C. K. Bowie¹

¹Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, 3101 Valley Life Sciences Building, Berkeley, CA 94720, USA

²Prizker Laboratory for Molecular Systematics and Evolution, The Field Museum, 1400 South Lake Shore Drive, Chicago IL 60605, USA

³Department of Wildlife and Fisheries Sciences, Biodiversity Research and Teaching Collection, Texas A&M University, College Station, TX 77843, USA

ABSTRACT

The Fiscal Flycatcher, *Sigelus silens*, is the only representative of a monotypic genus, endemic to Southern Africa, and may represent two cryptic species. Here we describe the development of thirteen microsatellite markers, and characterize polymorphism for each one. We found that all but one of our 13 loci were highly variable, each having five or more alleles. This suggests that these markers will have high variability across the species range and will be of utility in understanding the extent of gene flow among populations.

Keywords: Africa, Muscicapidae, Passeriformes

The Fiscal Flycatcher *Sigelus silens* is the only representative of a monotypic genus, and endemic to Southern Africa. Two subspecies are recognized and paleoclimate models suggest that these taxa may have been restricted to distinct refugia in the past (Bowie et al. Unpubl. Data), and hence may represent cryptic species. The Fiscal Flycatcher is unusual in that it closely mimics the plumage of the Common Fiscal Shrike (*Lanius collaris*) a predatory songbird species widespread across much of southern Africa. In order to examine the extent of population structure within the Fiscal Flycatcher, and to better understand the population trends and evolutionary history of this species, as well as to generate a framework for the comparison of phylogeographic and

landscape genetics patterns between the Fiscal Flycatcher and the Common Fiscal Shrike, we developed thirteen tetra or penta-nucleotide microsatellite markers.

Microsatellite enrichment followed the standard protocol of Glenn and Schable (2005). Genomic DNA from a single individual was digested with *RsaI* and *XmnI*, and SuperSNX24 linkers were ligated onto the resulting DNA fragments. Five biotin labeled tetranucleotide probes were hybridized to the fragmented DNA: (AAAT)₈; (AACT)₈; (AAGT)₈; (ACAT)₈; (AGAT)₈. The probe-DNA complex was attached to streptavidin-coated magnetic beads (Dynabeads M-270, Invitrogen) and washed twice with 2X SSC, 0.1% SDS and four times with 1X SSC, 0.1% SDS at 52°C. Enriched fragments were separated from the beads through denaturing and standard ethanol precipitation. In total, 88 colonies were sequenced, 69 of which contained repetitive elements.

Enriched fragments that had tetra or penta -nucleotide repeats with at least six but preferably eight repeats were selected for additional processing. Primers were designed for 18 loci in WebSat (Martins et al. 2009). Each forward primer was 5' tagged with either the HEX or FAM flourophore.

We sampled 23 *Sigelus silens* from South Africa. Blood samples were stored frozen in lysis or DMSO/NACK buffer until extraction using the manufacturer's protocol for the DNeasy Kit (Quigen, USA). Loci were PCR-amplified in 10µl reactions consisting of 1µl DNA (diluted 1:10), 0.12U of Taq polymerase (Invitrogen), 1µl 10×PCR buffer, 0.3µl 50mM MgCl₂, 0.6µl 10 µg/µL BSA, 0.25µl 10mM dNTPs and 0.6µl 10mM of each primer. Thermocycling conditions for all markers were: initial denaturation at 94°C for 3 minutes, then 30 cycles consisting of denaturation at 94°C for 45s, annealing at primer specific temperature for 30s, and extension at 72°C for 45s,

followed by a final 30 minute extension at 72°C. Fragments were suspended in formamide and analyzed using LIZ500 then genotyped on an ABI-3730. We used Genemapper 4.0 (Applied Biosystems, USA) to size and bin the alleles. All individuals were PCR-amplified and genotyped twice to ensure marker consistency and repeatability.

We assessed repeat motif, number of alleles, size range of alleles, expected and observed heterozygosity (with 1000 replicates), and polymorphism information content (PIC) in the PopGenKit R package (Paquette 2013). Departures from Hardy-Weinberg Equilibrium (HWE) were assessed using the exact test in the pegas R package (Paradis 2010). Micro-checker was used to check for the presence of null alleles (van Oosterhout et al. 2004).

Of thirteen microsatellites, twelve were highly variable. Observed heterozygosity values ranged from 0.043 to 0.87 and the number of alleles ranged from two to eleven (Table 1). PIC values indicate that twelve loci were highly polymorphic with high information content (Table 1). The population examined here is in HWE, but the exact test suggests that three loci exhibit a departure: SSI2, SSI24, and SSI34. There was no evidence for scoring errors, although locus SSI2 had a general excess of homozygous individuals, suggesting that it may contain null alleles.

The microsatellite loci developed and characterized in this study will be of utility in assessing the nature of gene flow among populations of this endemic Southern African flycatcher, and will provide insight into the effect of landscape features on population structure.

Acknowledgements

The authors thank Hanneline Smit, Graeme Oatley, Ângela Ribeiro, Dawie de Swardt and Jérôme Fuchs for help with collecting samples. This research was supported by NSF grants to R.C.K.B and G.V. (DEB1120356, DEB1119931). This is publication XX of the Biodiversity Research and Teaching Collections at Texas A&M.

References

- Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci *Methods in Enzymology* 395:202-222
- Martins WS, Lucas DCS, Neves KFS, Bertoli DJ (2009) WebSat- a web software for microsatellite marker development *Bioinformatics* 3:282-283
- Paquette S (2013) PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets. CRAN,
- Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach *Bioinformatics* 26:419-420
- van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data *Molecular Ecology Notes* 4:535-538

Locus	Genbank Accession	Primer Sequence (5'-3'), fluorescent label	Repeat motif	T _a (°C)	Size Range (bp)	N _A	H _O	H _E	HWE (p-value)	PIC
SSI 2	KJ580508	F-(FAM)-TTTCTGATTCTTAGGCCACTGA R-CTATAAACCACAGCTCCTTCCC	(ATAG) ₁₆	52	147-175	8	0.609	0.834	0.028	0.813
SSI 6	KJ580509	F-(FAM)-TCTGGTAACTTGACTCTGAAGAATG R-GAATAACTGCCCTGTTCCCTTG	(ATAG) ₉	59	333-353	6	0.696	0.703	0.264	0.669
SSI 8	KJ580510	F-(HEX)-AGCCGTGTCTTATAGATGGGAA R-AATCACCACCTTCTTGCTGAGGT	(ACAT) ₁₄	56	256-274	6	0.739	0.787	0.737	0.754
SSI 9	KJ580511	F-(FAM)-AGCAGTCATTCTCAGTGGAGGT R- CAGCAAGGAACAGGTTACACAC	(ATCC) ₁₃	52	188-224	10	0.87	0.834	0.667	0.813
SSI 14	KJ580512	F-(FAM)TTTGTATGGCCCTGTCTTAGT R-GCTCTCTGGTGGAAGTATTGG	(TATC) ₉	56	254-274	7	0.783	0.833	0.361	0.811
SSI 17	KJ580513	F-(HEX)-GCTTGGCTAATCTGAAATGTGA R- GTAGGTAGGAAGGACAAGTCC	(TATC) ₁₃	53	285-333	9	0.826	0.832	0.509	0.814
SSI 20	KJ580514	F-(FAM)-CACCTGGAGAGAAGAAAAGAGAA R- TGACTGTTGCTGAAGGCATATAG	(TATC) ₁₀	56	263-271	5	0.696	0.768	0.576	0.726
SSI 22	KJ580515	F-(HEX)-CTTGGTTCACTCTCAACACCTG R-GCAGAATCACAAACACTTGGTC	(TATC) ₁₁	53	154-186	7	0.783	0.809	0.369	0.782
SSI 24	KJ580516	F-(FAM)-ATTCCCGTCTGTTCCCTCTA R- GCTGTCTGTAGGAGCAGTGAGA	(ATAG) ₁₇	58	206-226	8	0.652	0.813	0.001	0.789
SSI 29	KJ580517	F-(FAM)-CAACATACCAGAGCACAGAAA R-AGGGCTTGACCTAAGCTGTTAAT	(TCTA) ₁₂	64	372-394	8	0.773	0.823	0.053	0.801
SSI 33	KJ580518	F-(FAM)-TGAACTCCCCTAATTCAGGGT R-GACCTCCGATCTTACAAACTGG	(ATGG) ₁₅	58	320-357	9	0.87	0.837	0.400	0.816
SSI 34	KJ580519	F-(HEX)-GATGTTTCCCTTCCATCAAAGA R-AAGCCACATGGTATCAAAAAGGT	(TACCA) ₁₄	53	187-217	11	0.783	0.886	0.024	0.875
SSI 40	KJ580520	F-(HEX)- GAATCACCATCACTTCAACCAA R- CCATCCCAGGAGACAAGAGTAA	(TATG) ₆	51	384-392	2	0.043	0.043	1.000	0.042

Table 1. Characteristics of microsatellite loci for *Sigelus silens*

ACAACACCCAAGTGGTGAGTGCAGAGAACAGCAATTCTGACTGAGAGCAGCAATTCTGACTGAGACATGATCC
CACCCAATCCTGGCAAATGGACCTCCCCAGTTCTAACACACATTACAGCAGCATCCTTCTAACTATGAAAATCT
ATTTATTATTAGGTGTAGCCACAAAGAAACATGCAAATATCTTCCATGTGCCTGTATTAATATTGTTTATTA
ATTTCTTTTTTTCATTTAAGGCTTTGGTTTTGTTTTATGGCAGCTAGCAGTATATTTTTCTTTTTAAAATGAGA
ACCACACCAAACAGAGTCACACCGACCCTCAAAGACAATTTGCATGTGCTATAAAAAAGCAGAAATCTTTTTTA
TTGGTTAATCATAAATGTAGTATTGTAGGTTTCTGCCTAAAAACAGCTCAACATAACCAGAGCACAGAAAAAA
AAAATCTGAAAAAGAAGAAAGTATGGAAAAAAGCATATATATGTCCTATATACCTAGGTTATATAGACATA
TATCTATATAACATATAGACATACATATCTATATATCTAGGTAATCTATCTATCTATCTATCTATCTAT
CTATCTATCTATCTATCTACCTCCCCATAACCAGGAAGTAGTGGCTTTTTAAAACATAATTGTCTATTAGAAAG
CCAAACATTTGTAATTCATTAGAAAAGTCCAAAATAGATAATTATTATAAGACCATACAACAGTATTTGATC
AACTATACTAACTCAAATAACACTATTCTGAGACTTTTTAAAGAACATTAACAGCTTAGGTCAAGCCCTGCAA
AATCACCAGGTTACTAAAGGGCTCATCCTATTCTGACACAAAGAAATTCTTGTAACCTGAGATATGTTCTGC
TTAATTTCTTAAACAGGAACATTTCTGTGAAATGCCAGACTGGGTCTGCTGAGCTCCCCACGCTGCCTCATCTGC
TCAGGGGATGTTGAAATGTATTTTATAGGTAATGAACTCCACACTGAGCCATGGCATCTCCCTCATCTTTC
TGGTCTCTAACTACTCTGCTGATGTTTCTTTCACATTTTAGCGGTAAGGCCTATGGGACCAGGACCAGGGAAT
GCTTTACTCATAGTGATCCTGAGCAATGAATCATGAATGCTCTGCTCCCAAAAATAATTTAGCATTCCCTTGTA
ACAAATGAGAGATCTTCTATTGATTTTTCAGCAAAGCCTTTCTCCCTCTCTCTTCAGCCTCACTCTGCACTTCTGT
CCAAATACCTGATTGACTGATGCTCCACACATCCCTTCCCCTGGTATGTCATAGCAGAAGAAAGAAACACAG
ATTTTCAAATAAAAATATGCAAATGAGAACAGCTTTGCCTAGGTCTGCTCAGATTTCTGAACTCCTTTTTT
TATGGCAGCCTGTATTTAATTTTTTTTTCTGGGGATCGTAAAGCTGAAAAGATTCTGCTAGCTAGGCCTTAA
CAA

>SSI33

ACAATACCTGATATCTTCTCTGTTATTGTTGAAGATAAAGCCCACAAGACCCACCCCTGTAAATAAAATCTGT
ATGTCACAAATTAGAGCAAGCAGTTTCTCATTATGAATAATTCGTGTTCCCTTCCATGACTTCTCACTGACAGA
ACTTAGAGTTCCCTCTGTTTCATTCAAAGAGAAAATGTGTTCACTCAACTTTCCTGGCTTGAACCTCCACTAA
TTCAGGGTAAGAGCAATGTGAAGCAGAGATCCTGTATCACTCATTTCAGTAGTAGGCAGCTCCCAAATGGGAGA
AGCCAAGGTGATTCCAGTTATGTTAAAGCAGCTTACACTAGAAGATGGATGGATGGATGGATGGATGGATG
GATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATGGATG
GATAAAAACCAAGGATGCAACAATGTTTCTTGAACATTATTTTTAAAAGGCTTTTGAGTTGCTTTTTTATTAA
GTAGATCCAGTTTGTAAAGATCGGAGGTCAAGATAAATATTTAAAAGTATATATAAATATGTAGCTCCATAGG
AGTTGCTAAAAGCTTCTGCTTCCATCTGCATATGGGCATAGGAATT

>SSI34

ACAAGGAGTTCTAGTCTGGAAAAGCAGAATGCAATTAGACATTTCTGTGGTTGGCAAAGTGCAGCAAGGACT
AAGCTGGGATATCAAAACACATTGCTGAAGAACAGTGTGCTTATATCATGTGTGTTATAGGTTTGGATTTA
AAAACCTAAAGGGGATGTTTCCCTTCCATCAAAGAACTTTCATTGAACATAGATGTGGTAAGTGGCAGCTAA
ATACTTAATATGAAGATTACATACCATAACCATAACCATAACCATAACCATAACCATAACCATAACCATA
CCATAACCATAACCATAACCAGTTTGGATTTTTTCTCTGTAACACAAGGCAGTCAGAACTACCTTTTGATACCAT
GTGGCTTTATTACTTTTAGTTTACCAGTAGAGGAAAGCACTTAAAAGCAAACCATAAGATTCCCACAGTGT
CTTCTGTTTCAAAGGTCTGACTTGGATGAATTGCTGCACTGAAGTGAATTAATCACTAGCTGTGAGATGGA
ACAAGGTGGCTGGCATAGCAGATAGAAGAAAAATTGTCTGCAGGATGCACAGATGAATGGGCCATGTCTGG
AGGAGCACTAGTGTGCTGTCTTGTTCATAACACGAATTGTGTCTCCCTTTTGACTTCATCTCGAGTTCT
AGTAGTTTTTCAGGTGATGAGGGAGGCTGTCCCTGCTTTTGCAATCACTGTAAGTATTCTGGCTTTGCAGAAG
AGATAATGGTAAAGAGTAATGGTAGTATTTACTAATCATTTGTCTTTTCATTTTCATCCTCAGT

>SSI40

ACCATCACTTCAACCAAGGGACATCTAAGCTGCAATGATCAAGACTCATGGAATGTTGACAGGGTGGTGAGAT
TTTCATTCACTTTCTCTCTTTCTCTCTTTTCCCTTACACGAGTCTTGGGTGATACTTTTGTGCTTTCACATTA
CAAGGCTTTCATATTACTATAGATAATAACAATCAAACAACACTACATACCTGCTTTCTTTGCAACCAAA
TTGTTCTAAAATAAACATAATACATTTTTATATATGTGTGTGTATATGTATGTATGTATGTATGTATGT

ATTTACAAACATCAGTCATTCAGTGTGTCTCACTCTAATCCACTCCAAGAAATCTATTAACAAGAACTTGGG
TTACTCTTGTCTCCTGGGATGGATCATGACAAAGCACA