

1 **Isolation and characterization of 7 microsatellite loci for the Florida Burrowing Owl**

2 (*Athene cunicularia floridana*)

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21 **Abstract**

22 Despite long-term scientific interest in the Florida Burrowing Owl, little is known about key
23 aspects of its ecology that likely influence population persistence. This is a cause of alarm
24 considering that the Florida Burrowing Owl has been designated a “Species of Special Concern”
25 since 1979. Therefore, seven microsatellite loci were developed and characterized for 48 Florida
26 Burrowing Owls. All loci were polymorphic and six of them differed significantly from Hardy-
27 Weinberg expectations. Although preliminary, the number of loci out of HW equilibrium may
28 result from low population size and resultant non-random mating that could be caused by habitat
29 fragmentation as development has ensued.

30 **Introduction**

31 Historically, the Florida Burrowing Owl (*Athene cunicularia floridana*) occupied the
32 prairies of the central peninsula (Ridgway 1914; Bent 1938; Nicholson 1954), but expanded its
33 range southward toward the Atlantic and Gulf coasts in the 1940s and 1950s due to habitat
34 alteration stemming from residential development (MacKenzie 1944; Courser 1979, Millsap &
35 Bear 1997). Despite long-term scientific interest in the Florida Burrowing Owl, however, little is
36 known about key aspects of its ecology that likely influence population persistence. This is a
37 cause of alarm considering that the Florida Burrowing Owl has been designated a “Species of
38 Special Concern” since 1979 (Millsap 1997), because some highly visible urban populations
39 were extirpated.

40 Nearly 86% of Florida Burrowing Owls reside in the southeast and southwest regions of
41 the state. While residential development provides new, open habitat for Burrowing Owls,
42 persistence in these areas is fleeting as owl density declines abruptly when greater than 60% of
43 the land is developed (Wesemann 1986). Thus, Burrowing Owl populations in developing urban

44 areas of south Florida are in a state of flux between colonization, brief persistence, and
45 extirpation (Wesemann 1986; Millsap 2002).

46 In addition to demography and population dynamics, genetic variation can have profound
47 effects on population persistence (Frankham, Ballou & Briscoe, 2002). Reported low dispersal
48 distances (Millsap and Bear 1997) and high philopatry (Millsap & Bear 1997; Lutz & Plumpton
49 1999) in Burrowing Owls may promote inbreeding due to non-random mating (Millsap & Bear
50 1997; Lutz & Plumpton 1999). The potential problems associated with reduced genetic variation
51 at the population level include lowered resistance to infectious diseases and parasites, reduced
52 survival, and increased probability of extinction (Pemberton et al. 1988; Frankham 1995;
53 Frankham, Ballou & Briscoe, 2002; Newman & Pilson 1997; Thursz et al. 1997; Saccheri et al.
54 1998; Coltman et al. 1999). Therefore, colonization and extinction dynamics (Wesemann 1986;
55 Millsap 2002), low dispersal distances (Millsap and Bear 1997), and patchy distribution (Bowen
56 2000) of Florida Burrowing Owls may interact to accelerate extirpation of these populations.
57 Therefore, we describe the development of seven microsatellite markers that can be used to
58 assess levels of genetic variation of Florida Burrowing Owls.

59

60 **Materials and Methods**

61 All burrowing owls were captured and handled in accordance with guidelines of the
62 United States Department of the Interior, US Geological Survey (Permit Number 22418), Florida
63 Fish and Wildlife Conservation Commission (Permit Number WB03367), and the University of
64 South Florida Institutional Animal Use and Care Committee (Permit Number 2878).

65 Owls were captured using noose-carpet traps placed at burrow entrances (Millsap & Bear
66 1997). Approximately 200 μ L of blood was drawn from the wing vein (Wingfeld & Farner 1976;
67 Desmond 1997) of all captured individuals. Blood was stored in 5 ml EDTA blood tubes and
68 treated with "Easy Blood"- a buffer used for preserving blood at room temperature for later DNA
69 extraction. DNA was extracted from the blood of 48 owls using a Qiagen® Blood and Cell
70 Culture DNA Mini Kit.

71 For microsatellite discovery, five μ g of high quality burrowing owl DNA was sheared
72 into fragments of approximately 500bp lengths. Linkers were ligated onto the sheared DNA and
73 then quantified for a titration run on a Roche 454 pyrosequencer. A titration was necessary to
74 establish the appropriate concentration of the sheared, labelled DNA that was needed in order to
75 generate high quality sequences before committing to a full 454 whole-genome sequencing run.
76 The sequence data were mined for di-, tri-, and tetra- nucleotide repeats using a Pearl script
77 search. The sequences were visually examined to determine the length of the flanking sequences
78 and the potential for appropriate primer sequences for PCR amplification. The unique sequences
79 were then submitted to Primer3 for primer design. The default parameters were left in place and
80 groups of sequence were limited in size to facilitate multiplexing.

81 A first-round PCR amplified the target region using 5' M13-tailed gene-specific forward
82 primers and gene-specific reverse primers, and a second round used a fluorescent WellRED D2,
83 D3, or D4 dye-labeled M13 forward primer and unlabeled gene-specific reverse primer.
84 Touchdown PCR conditions for the initial and second amplification were identical. Initial
85 denaturation was 94°C for two min, followed by a second denaturation at 94°C for 15 sec,
86 annealing for 15 sec, and extension at 68°C for 30 sec. The annealing temperature decreased by

87 1⁰C, from 58⁰C to 56⁰C, for cycles 1-3, 54⁰C for three cycles, and 52⁰C for the final twenty-five
88 cycles.

89 PCR products were separated and sized on a Beckman GeXP Genetic Analysis System.
90 Alleles were scored using the Soft Genetics Gene Marker software (Version 2.6.4). The number
91 and range of alleles at each locus was calculated using GenAlEx (Version 6.41) (Peakall &
92 Smouse 2006). We then tested for Hardy–Weinberg expectations (HWE) at each locus using
93 exact tests in GenAlEx.

94

95 **Results**

96 We originally amplified 14 loci, but only those in which $\geq 75\%$ of the samples amplified
97 were included in the analysis. All loci were polymorphic (Table 1). No more than 5 alleles were
98 observed at any locus, while ≤ 3 alleles were observed at the majority (71%, N=5) of loci. Four
99 loci differed significantly from Hardy-Weinberg expectations.

100

101 **Discussion**

102 The data are indicative of a population that exhibits low genetic variation. Habitat
103 fragmentation, as a result of unabated development, is a possible indirect influence on the
104 number of loci out of HW equilibrium. Likely drivers of HW disequilibrium include low
105 population size and non-random mating. Adult Florida Burrowing Owls in some populations
106 exhibit year-round site fidelity and do not disperse (Millsap & Bear 1997; N. Ritchie
107 pers.comm.). Philopatry (i.e. the process whereby dispersing organisms settle and reproduce
108 close to their birth site) resulting from short dispersal distances of juvenile males appears to
109 promote incestuous (mother-son) matings (Millsap & Bear 1997). The potential problems

110 associated with reduced genetic variation due to inbreeding include lowered resistance to
111 infectious diseases and parasites, reduced survival, and increased probability of extirpation
112 (Pemberton et al. 1988; Frankham 1995; Frankham, Ballou & Briscoe 2002; Newman & Pilson
113 1997; Thursz et al.1997; Saccheri et al. 1998; Coltman et al. 1999). We are planning to initiate a
114 state-wide genetic survey of Florida Burrowing Owls and these novel microsatellites primers will
115 be useful for more in-depth genetic analyses.

116

Table 1. Characterization of *Athene cunicularia floridana* microsatellites (N_a , number of alleles; N_e , effective number of alleles, H_o , observed heterozygosity; H_e , expected heterozygosity; P_{HWE} , probability of deviation from Hardy-Weinberg equilibrium).

Locus	Primer Sequence	Repeat Motif	Tm	Size Range	N_a	N_e	H_o	H_e	P_{HWE}
FLBUOW-3	CAAGCCAGCTATCGCGTTG TGCATGTGCATGGAATTAGCTC	(CA) ₁₉	58-52	248-272	4	1.94	0.44	0.48	<0.001
FLBUOW-4	TGTGAGTAAGTGTCAGGAGC AGGCAGATGGTTCAGAGGTG	(GT) ₁₇	58-52	211-217	3	2.76	0.92	0.64	<0.001
FLBUOW-5	ACTGCCATGGTGTTCAC ACAACGGTGTACTAGGGACTG	(TA) ₁₆	58-52	206-210	3	2.50	0.47	0.60	0.141
FLBUOW-7	ACTGCCATGGTGTTCAC CGGATGTATGGAGGTCGCC	(TA) ₁₄	58-52	322-326	3	2.57	0.46	0.61	0.153
FLBUOW-9	ACACCTTACACAATCCTGTGC AGCTGACCAGAGGAGTATTGC	(CA) ₁₃	58-52	170-174	3	1.07	0.02	0.06	<0.001
FLBUOW-11	TCTAGTCGGTGCCAACATC CTGACAACACAAGCTGCCC	(CA) ₁₀	58-52	109-209	5	2.78	0.69	0.64	<0.001
FLBUOW-14	GTTCCACATCTGCAAGTCAG TGGCCACCATACAGATGCC	(TG) ₁₀	58-52	287-289	2	1.29	0.26	0.22	0.316

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125

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