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

(Athene cunicularia floridana)

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Abstract

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

Introduction

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

Nearly 86% of Florida Burrowing Owls reside in the southeast and southwest regions of the state. While residential development provides new, open habitat for Burrowing Owls, persistence in these areas is fleeting as owl density declines abruptly when greater than 60% of the land is developed (Wesemann 1986). Thus, Burrowing Owl populations in developing urban
areas of south Florida are in a state of flux between colonization, brief persistence, and extirpation (Wesemann 1986; Millsap 2002).

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

Materials and Methods

All burrowing owls were captured and handled in accordance with guidelines of the United States Department of the Interior, US Geological Survey (Permit Number 22418), Florida Fish and Wildlife Conservation Commission (Permit Number WB03367), and the University of South Florida Institutional Animal Use and Care Committee (Permit Number 2878).
Owls were captured using noose-carpet traps placed at burrow entrances (Millsap & Bear 1997). Approximately 200 uL of blood was drawn from the wing vein (Wingfeld & Farner 1976; Desmond 1997) of all captured individuals. Blood was stored in 5 ml EDTA blood tubes and treated with "Easy Blood"- a buffer used for preserving blood at room temperature for later DNA extraction. DNA was extracted from the blood of 48 owls using a Qiagen® Blood and Cell Culture DNA Mini Kit.

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

A first-round PCR amplified the target region using 5’ M13-tailed gene-specific forward primers and gene-specific reverse primers, and a second round used a fluorescent WellRED D2, D3, or D4 dye-labeled M13 forward primer and unlabeled gene-specific reverse primer. Touchdown PCR conditions for the initial and second amplification were identical. Initial denaturation was 94°C for two min, followed by a second denaturation at 94°C for 15 sec, annealing for 15 sec, and extension at 68°C for 30 sec. The annealing temperature decreased by
1°C, from 58°C to 56°C, for cycles 1-3, 54°C for three cycles, and 52°C for the final twenty-five cycles.

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

Results

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

Discussion

The data are indicative of a population that exhibits low genetic variation. Habitat fragmentation, as a result of unabated development, is a possible indirect influence on the number of loci out of HW equilibrium. Likely drivers of HW disequilibrium include low population size and non-random mating. Adult Florida Burrowing Owls in some populations exhibit year-round site fidelity and do not disperse (Millsap & Bear 1997; N. Ritchie pers.com.). Philopatry (i.e. the process whereby dispersing organisms settle and reproduce close to their birth site) resulting from short dispersal distances of juvenile males appears to promote incestuous (mother-son) matings (Millsap & Bear 1997). The potential problems
associated with reduced genetic variation due to inbreeding include lowered resistance to
infectious diseases and parasites, reduced survival, and increased probability of extirpation
(Pemberton et al. 1988; Frankham 1995; Frankham, Ballou & Briscoe 2002; Newman & Pilson
1997; Thursz et al. 1997; Saccheri et al. 1998; Coltman et al. 1999). We are planning to initiate a
state-wide genetic survey of Florida Burrowing Owls and these novel microsatellites primers will
be useful for more in-depth genetic analyses.
Table 1. Characterization of *Athene cunicularia floridana* microsatellites (*Nₐ*, number of alleles; *Nₑ*, effective number of alleles, *H₀*, observed heterozygosity; *Hₑ*, expected heterozygosity; *PₑWₑ*, probability of deviation from Hardy-Weinberg equilibrium.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Sequence</th>
<th>Repeat Motif</th>
<th>Tm</th>
<th>Size Range</th>
<th><em>Nₐ</em></th>
<th><em>Nₑ</em></th>
<th><em>H₀</em></th>
<th><em>Hₑ</em></th>
<th><em>PₑWₑ</em></th>
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</thead>
<tbody>
<tr>
<td>FLBUOW-3</td>
<td>CAAGCCAGCTATCGCGTTG TGCATGTGCATGGAATTAGCTC</td>
<td>(CA)₁₉</td>
<td>58-52</td>
<td>248-272</td>
<td>4</td>
<td>1.94</td>
<td>0.44</td>
<td>0.48</td>
<td>&lt;0.001</td>
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<tr>
<td>FLBUOW-4</td>
<td>TGTGAGTAAGTGTCAGGAGC AGGCAGATGGTTTACAGAGGTTG</td>
<td>(GT)₁₇</td>
<td>58-52</td>
<td>211-217</td>
<td>3</td>
<td>2.76</td>
<td>0.92</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FLBUOW-5</td>
<td>ACTGCCATGGTGTGTTGAC ACAACGGTTGACTAGGGACTG</td>
<td>(TA)₁₆</td>
<td>58-52</td>
<td>206-210</td>
<td>3</td>
<td>2.50</td>
<td>0.47</td>
<td>0.60</td>
<td>0.141</td>
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<td>FLBUOW-7</td>
<td>ACTGCCATGGTGTGTTGAC CGGATGTATGGAGGAGTGC</td>
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<td>58-52</td>
<td>322-326</td>
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<td>2.57</td>
<td>0.46</td>
<td>0.61</td>
<td>0.153</td>
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<tr>
<td>FLBUOW-9</td>
<td>ACACCTTACAAATCCTGTCAG GTGACCAGAGGATATTG</td>
<td>(CA)₁₃</td>
<td>58-52</td>
<td>170-174</td>
<td>3</td>
<td>1.07</td>
<td>0.02</td>
<td>0.06</td>
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<tr>
<td>FLBUOW-11</td>
<td>TCTAGTGGTGCCAACCATC CTGACAACAAACAGGAGCTGCC</td>
<td>(CA)₁₀</td>
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<td>109-209</td>
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<td>FLBUOW-14</td>
<td>GTCCCCACATCTGCAAGTCAG TGGCCACCACATACAGATGCC</td>
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<td>58-52</td>
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<td>2</td>
<td>1.29</td>
<td>0.26</td>
<td>0.22</td>
<td>0.316</td>
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</table>
Acknowledgments

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References


