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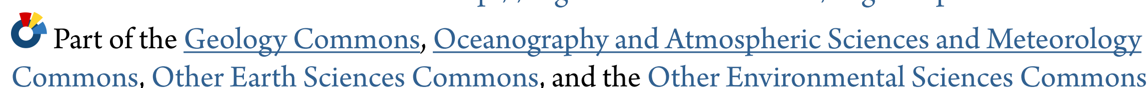
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**PRIMER NOTE**

# Microsatellite loci for distinguishing spotted owls (*Strix occidentalis*), barred owls (*Strix varia*), and their hybrids

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### Abstract

**We identified four diagnostic microsatellite loci that distinguish spotted owls (*Strix occidentalis*), barred owls (*Strix varia*), $F_1$ hybrids and backcrosses. Thirty-four out of 52 loci tested (65.4%) successfully amplified, and four of these loci (11.8%) had allele sizes that did not overlap between spotted and barred owls. The probability of correctly identifying a backcross with these four loci is 0.875. Genotyping potential hybrid owls with these markers revealed that field identifications were often wrong. Given the difficulty of identifying hybrids in the field, these markers will be useful for hybrid identification, law enforcement and spotted owl conservation.**

*Keywords*: backcross, $F_1$, hybrid identification, microsatellites, *Strix occidentalis*, *Strix varia*

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The northern spotted owl (*Strix occidentalis caurina*) is a threatened subspecies under the US Endangered Species Act (ESA) associated with rapidly declining, old forests in western North America (US Fish & Wildlife Service 1990). An additional threat to northern and California spotted owls (*Strix occidentalis occidentalis*) comes from competition and hybridization with barred owls (*Strix varia*), a close relative historically occurring in eastern North America which has rapidly expanded its range westward into that of northern and California spotted owls. Since hybrid owls are not protected under the ESA, it is critical for law enforcement officials to be able to identify pure northern spotted owls, which are legally protected.

As field identification of spotted owl × barred owl hybrids is difficult, an amplified fragment length polymorphism (AFLP) method was developed for hybrid identification (Haig *et al*. 2004). A limitation of AFLPs, however, is that they cannot positively distinguish $F_1$s from backcrosses because they are dominant markers. Distinguishing between $F_1$s and backcrosses is important for determining the nature and extent of hybridization and for making management decisions. In this study, we identified microsatellite markers that distinguish spotted owls, barred owls, $F_1$ hybrids and backcrosses because of their codominant inheritance.

We identified diagnostic microsatellite markers using a three-step process. First, we tested which of 52 previously developed candidate loci successfully amplified in a subset of spotted owls ($n = 7$). For those loci that amplified, we then examined allele size variation in a subset of spotted owls ($n = 7$), barred owls ($n = 6$) and $F_1$ hybrids ($n = 2$) to identify potentially diagnostic markers with non-overlapping allele sizes. Finally, we verified that allele sizes did not overlap between spotted and barred owls for these markers using larger samples of spotted owls ($n = 49$), barred owls ($n = 29$) and hybrids ($n = 6$). Spotted owl samples included northern spotted owls (*S. o. caurina*) from Washington, Oregon and California ($n = 10$ from each state); California spotted owls (*S. o. occidentalis*) from California ($n = 10$); and Mexican spotted owls (*Strix occidentalis lucida*) from Arizona ($n = 9$). Barred owl samples were from Wisconsin ($n = 7$), Texas ($n = 2$), Washington ($n = 4$), Oregon ($n = 12$), and California ($n = 4$). Hybrids were from Oregon. Owls were identified in the field based on plumage characteristics, vocalizations and/or matings between known parents. We also examined an additional six potential $F_1$ hybrids and potential backcrosses from Oregon ($n = 5$) and Washington ($n = 1$) for which taxonomic status was unresolved by field biologists.

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**Table 1** Microsatellite loci for distinguishing spotted owls, barred owls and their hybrids

| Locus | Repeat motif | Primer sequences (5′–3′) | $T_a$ (°C) | Allele sizes | |
|---|---|---|---|---|---|
| | | | | Spotted owls | Barred owls |
| Bb126 | $(GA)_{15}$ | F: TCTCCAGAAGGGTTGTCATC<br>R: TGCTAAAACCTTACAGAATAACAG | 45 | 183 | 179 |
| BOOW18 | $(AAAG)_6$ | F: TTCTGCCTTACAGTCTAGT<br>R: AAAAACCTATCTTTGTGAGT | 58 | 203 | 187, 193, 195, 197 |
| Oe045 | $(GATA)_{13}GATTA(GATA)_{10}$ | F: GTATGTTCTACGTTGGATTTCCA<br>R: AAACCTGGCAAGTGCTGTT | 58 | 125, 129 | 113 |
| Oe128 | $(GATA)_{13}$ | F: CGTTGTAAATGATGAATCGCCTAGTGC<br>R: ATGCATGTATACATACAAACCTGG | 64 | 307, 311, 315, 319, 323, 327, 331 | 287, 291, 295, 299 |

$T_a$, annealing temperature. Repeat motifs are from the original sequenced clones. Bb126 primers are from Isaksson & Tegelström (2002), BOOW18 primers are from Koopman *et al*. (2004), and Oe primers are from Hsu *et al*. (2003; GenBank Accession nos are found in these references). Some alleles at BOOW18 differ in size from each other by increments of two bp in barred owls, indicating the presence of dinucleotide repeats at this locus despite a tetranucleotide repeat motif in the original boreal owl clone.

DNA from blood samples was isolated using standard phenol–chloroform extractions. DNA from muscle, heart and toe pads was isolated using DNeasy Tissue Kits (QIAGEN). The 52 loci initially screened were developed for spotted owls (six loci; Thode *et al*. 2002), eagle owls (*Bubo bubo*; seven; Isaksson & Tegelström 2002), Lanyu scops owls (*Otus elegans botelensis*; 18; Hsu *et al*. 2003, 2006), boreal owls (*Aegolius funereus*; seven; Koopman *et al*. 2004), burrowing owls (*Athene cunicularia*; seven; Korfanta *et al*. 2002), and ferruginous pygmy owls (*Glaucidium brasilianum*; seven; Proudfoot *et al*. 2005). Reaction mixtures (10 μL total) for the four diagnostic loci (Bb126, BOOW18, Oe045 and Oe128; Table 1) contained approximately 20 ng of owl DNA, 0.4–0.5 U *Taq* DNA polymerase (Promega), 1 μL 10× polymerase chain reaction (PCR) buffer (Promega), 0.5–1 μL of 25 mm $MgCl_2$, 0.3–0.5 μL of 10 mm dNTPs, and 0.15–0.2 μL of each 20 μm primer. The forward primer of each primer pair was fluorescently labeled with FAM or VIC. Thermal cycling conditions included an initial 94 °C denaturation step for 7 min; 35 cycles at 94 °C for 30 s, the appropriate annealing temperature (Table 1) for 45 s, and 72 °C for 45 s; and a final elongation step at 72 °C for 6 min. PCR fragments were analysed on an ABI 3100 capillary DNA sequencer and results were processed using ABI GENESCAN and GENOTYPER software.

Thirty-four of 52 candidate loci (65.4%) successfully amplified using spotted owl DNA. Of these 34 loci, four (11.8%) had allele sizes that did not overlap between spotted and barred owls and showed expected genotypes for known $F_1$ hybrids and $F_1$ × barred owl backcrosses. Hardy–Weinberg (HW) proportions and linkage disequilibrium (LD) were not tested because many loci were invariant within species (in which case it is not possible to test for HW proportions or LD) and because samples were taken from different populations (in which case deviations from HW proportions and LD are expected even in the absence of null alleles and linkage). Nonetheless, alleles from the parental species segregated independently among loci in the three backcrosses identified, indicating that these loci are likely not tightly linked.

All owls identified as spotted owls in the field were also identified as spotted owls with the diagnostic microsatellite markers and all birds identified as barred owls in the field were confirmed as barred owls (Table 2). Likewise, all birds confidently identified as $F_1$ hybrids or backcrosses in the field were verified with microsatellites. However, five out of six birds identified as potential $F_1$s or backcrosses in the field were identified incorrectly. Of the four birds thought to be potential $F_1$s, one was a spotted owl and three were barred owls. The bird identified as a potential $F_1$ × spotted owl backcross was a pure spotted owl. Only the bird identified as a potential $F_1$ × barred owl backcross was correctly identified.

With four diagnostic loci, there is a probability of $0.5^4 = 0.0625$ that an $F_1$ × barred owl backcross would only have barred owl alleles at all four loci, and therefore be incorrectly identified as a pure barred owl. The same calculation applies to $F_1$ × spotted owl backcrosses. There is also a probability of 0.0625 that a backcross would have one allele from each parental species at all four loci, and therefore be incorrectly identified as an $F_1$ hybrid. Thus, the probability of correctly identifying a backcross with four loci is 1–0.0625–0.0625 = 0.875. These loci therefore provide a fairly high level of power for identifying backcrosses (as well as a probability of 1.0 of correctly identifying $F_1$ hybrids). Given the difficulty of correctly identifying spotted owl × barred owl hybrids from plumage, as illustrated above by incorrect field identifications, these markers will play a useful role in hybrid identification and spotted owl conservation.

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**Table 2** Identification of spotted owls, barred owls and their hybrids with diagnostic microsatellite markers

| | Microsatellite identification | | | |
|---|---|---|---|---|
| Field identification | Spotted owl | Barred owl | $F_1$ hybrid (SO × BO) | Backcross ($F_1$ × BO) |
| Spotted owl | 49 | 0 | 0 | 0 |
| Barred owl | 0 | 29 | 0 | 0 |
| $F_1$ hybrid (SO × BO) | 0 | 0 | 4 | 0 |
| Backcross ($F_1$ × BO) | 0 | 0 | 0 | 2 |
| Potential $F_1$ hybrid (SO × BO) | 1 | 3 | 0 | 0 |
| Potential backcross ($F_1$ × BO) | 0 | 0 | 0 | 1 |
| Potential backcross ($F_1$ × SO) | 1 | 0 | 0 | 0 |

SO, spotted owl; BO, barred owl. Potential $F_1$ hybrids and potential backcrosses were owls for which taxonomic status was unresolved by field biologists.


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