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USE OF DNA ANALYSIS TO IDENTIFY SEX OF NORTHERN SPOTTED OWLS (*STRIX OCCIDENTALIS CAURINA*)

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ABSTRACT.—The spotted owl (*Strix occidentalis*) is a monochromatic species with slight sexual size dimorphism in adults. Methods currently available to identify sex of adult owls are ineffective for juveniles. Blood samples taken from owls from the eastern Cascade Mountains, Washington were used for cDNA cloning of a Z- and W-linked gene, DZWM1, to identify the sex of adult owls of known sex. A blind assessment resulted in the correct identification of sex for all 59 owls sampled (45 subadult/adult and 14 juveniles recaptured as subadult/adult). We believe this technique can be used to identify the sex of owls that cannot otherwise be identified using less invasive morphometric methods. Both field and laboratory procedures are described.

KEY WORDS: *DNA*; *sex identification*; *spotted owl*; Strix occidentalis.

Uso de análisis de DNA para identificar sexo en *Strix occidentalis caurina*

RESUMEN.—*Strix occidentalis* es una especie monocromática con escaso dimorfismo sexual en el tamaño adulto. Los métodos comunmente disponibles para identificar sexo en los búhos adultos no son efectivos para juveniles. Muestras de sangre obtenidas de búhos del este de las Montañas Cascada, Washington, fueron usadas para clonación de cDNA de un gen Z-y W-ligado, DZWM1, para identificar el sexo de individuos ya determinados. Estas medidas resultaron en la identificación correcta del sexo para los 59 búhos muestreados (45 subadultos/adultos y 14 juveniles recapturados como subadultos/adultos). Creemos que esta técnica puede ser usada para identificar el sexo de búhos que, de otra manera, no podrían ser identificados usando métodos morfométricos menos invasivos. Tanto el procedimiento de campo como el de laboratorio son descritos.

[Traducción de Ivan Lazo]

The spotted owl (*Strix occidentalis*) is a monochromatic species with slight sexual size dimorphism in adults (Blakesley et al. 1990). Morphometric models and behavioral clues, such as vocalizations, have been used to identify the sex of adult spotted owls (Forsman et al. 1984, Blakesley et al. 1990). However, none of these methods are useful in identifying sexes of juvenile birds. It may be important to correctly sex juvenile spotted owls because the survival rate of juveniles is a significant element of models used to estimate the status of populations (Burnham et al. 1994). For example, information on habitats used by juveniles during dispersal can provide data on the effects of landscape conditions on age- and/or sex-specific survival.

Both hormone immunoassay and genetic analysis have been used to identify sex of birds. Two steroid hormone immunoassay methods have been developed and used for a number of species, but the level of accuracy varies among species (Bercovitz et al. 1978, Tell and Lasley 1991). Neither has

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been used in field conditions where it would be difficult to obtain multiple fecal samples from the same individual.

Genetic methods of sex identification such as chromosome analysis (karyotyping) and flow cytometry have been used with varying levels of success (Ivins 1975, Halverson et al. 1985, Prus and Schmutz 1987, Tiersch and Mumme 1993). Only Nakamura et al. (1990) and Valentine (1990) have reported high (99–100%) rates of correct sexing, using chromosome analysis and flow cytometry, respectively. In addition, analysis of nucleotide sequences of differential regions of avian sex chromosomes generally has only limited usefulness in diverse avian groups (Uryu et al. 1989, Quinn et al. 1990, de Kloet and de Kloet 1991, Longmire et al. 1991). Microsatellite probes can also be used to determine gender in a wide variety of avian species (Longmire et al. 1993).

Recently, cDNA cloning of a Z- and W-linked gene, DZWM1, from the domestic turkey (*Meleagris gallopavo*) has proven a reliable method to identify sex in psittacines and it has been increasingly tested successfully on many avian orders (Halverson 1990, Dvorak et al. 1992). Reliable procedures need to be developed for many species (Griffiths and Tiwari 1993, J. Longmire pers. comm.). Probes of this gene sequence are homologous to the differential region of both sex chromosomes and sex can be determined by restriction fragment length polymorphism.

The objective of this study was to determine the accuracy and utility of using cDNA cloning techniques to identify the sex of spotted owls. Our goal was to use this technique to verify the sex of juvenile owls to facilitate development of a noninvasive morphometric model for identifying the sex of juveniles under field conditions. Herein, we describe collection and analysis techniques and our results using DZWM1 to identify the sex of spotted owls.

METHODS

We collected blood samples from 45 subadult or adult spotted owls captured in the eastern Cascade Mountains of Washington in 1991–95. In addition, blood samples were collected from 308 juveniles, of which 14 were recaptured as subadults/adults on the study area. Owls were captured using techniques described by Forsman (1983); criteria for identifying sex of subadult and adult spotted owls are reported by Blakesley et al. (1990). Most blood samples were taken from members of pairs (19 of 20 females, 22 of 25 males).

Blood was drawn from the brachial vein of the wing after swabbing the area with alcohol disinfectant. Approximately 20 μl of blood was drawn using a 1.0 cc tuberculin syringe with a 22–25 ga needle. In 1991–92 the blood was flushed directly into a cryovial containing 70% ethanol to prevent contamination. In 1993–94, a heparinized capillary tube was used to collect 0.1–0.2 μl of blood. This technique did not involve direct withdrawal of blood. Rather, the vein was punctured and several drops of blood that accumulated on the surface of the skin were drawn into the capillary tube and then placed in the cryovial. Samples were labeled, refrigerated at 7°C for 2–10 d, then sent by mail to the laboratory for processing and identification of sex without information on the field identification of the owl.

Samples were centrifuged at 2000 rpm for 5 min in the initial collection tube. The supernatant was discarded and the residual pellet resuspended in DNA Isolation Buffer (0.1 M sodium chloride [NaCl], 0.05 M Tris pH 8, 0.1 M EDTA with 0.2 mg/ml Proteinase K and 0.5% SDS). The tubes were gently rocked at 55°C for 1–4 hr.

Following incubation, 0.5 μl saturated NaCl solution was added and the samples shaken vigorously for 15 sec. The samples were then centrifuged at 2000 rpm for 15 min and the supernatant decanted into a fresh tube, discarding the pellet. Three ml of 95% ethanol were added to the supernatant, and the solution gently mixed until the DNA precipitate formed. The precipitate was removed using a glass pipette hook, rinsed in 70% ethanol and allowed to air dry. The DNA was resuspended in TE (10 mM Tris pH 8, 2 mM EDTA) and dissolved by gentle rocking at 55°C for 1 d.

Twenty-five μl of sample (approximately 5 μg) was digested with SacI according to manufacturer's recommendations (Pharmacia Biotec, Piscataway, NJ, U.S.A.) in a total reaction volume of 35 μl. Ten μl of "Stop Dye" (Maniatis et al. 1982) was added and the samples loaded on a 0.8% agarose TBE gel. Electrophoresis was performed at 15°C with recirculating buffer at 45 amperes for 16–18 hr.

After electrophoresis, gels were photographed and trimmed. Gels were immersed in denaturant (1.5 M NaCl, 0.5 NaOH) for 30 min with gentle agitation, rinsed briefly with water, then immersed in neutralizer (1 M Tris pH 7.4, 1.5 M NaCl) for 30 min with gentle agitation. Gels were then set up for capillary blotting onto Hybond N (Amersham Corporation, 2636 South Clearbrook Drive, Arlington Heights, IL, U.S.A. 60005) with 10× SSC as the transfer buffer. Transfer was complete after 8–16 hr.

Blots were removed from the blotting apparatus, rinsed briefly in 5× SSC, and air dried. Blots were exposed for 2 min on a UV transilluminator to crosslink the DNA. Blots were then moistened in prehybridization fluid (0.5 M NaPhos pH 7.0, 7% SDS, 5 mM EDTA) and loaded into glass tubes with 5 ml of prehybridization fluid. The tubes were heated in the incubator for at least 15 min. The probe was added and hybridization proceeded for 12–18 hr at 65°C.

DNA analysis was performed as previously reported (Dvorak et al. 1992) except that restriction digests were performed with SacI.

Blots were washed twice in 2× SSC and 0.5% SDS for 15 min each at room temperature, then washed in 0.2× SSC, 0.5% SDS, for 25 min at 52°C. Blots were exposed

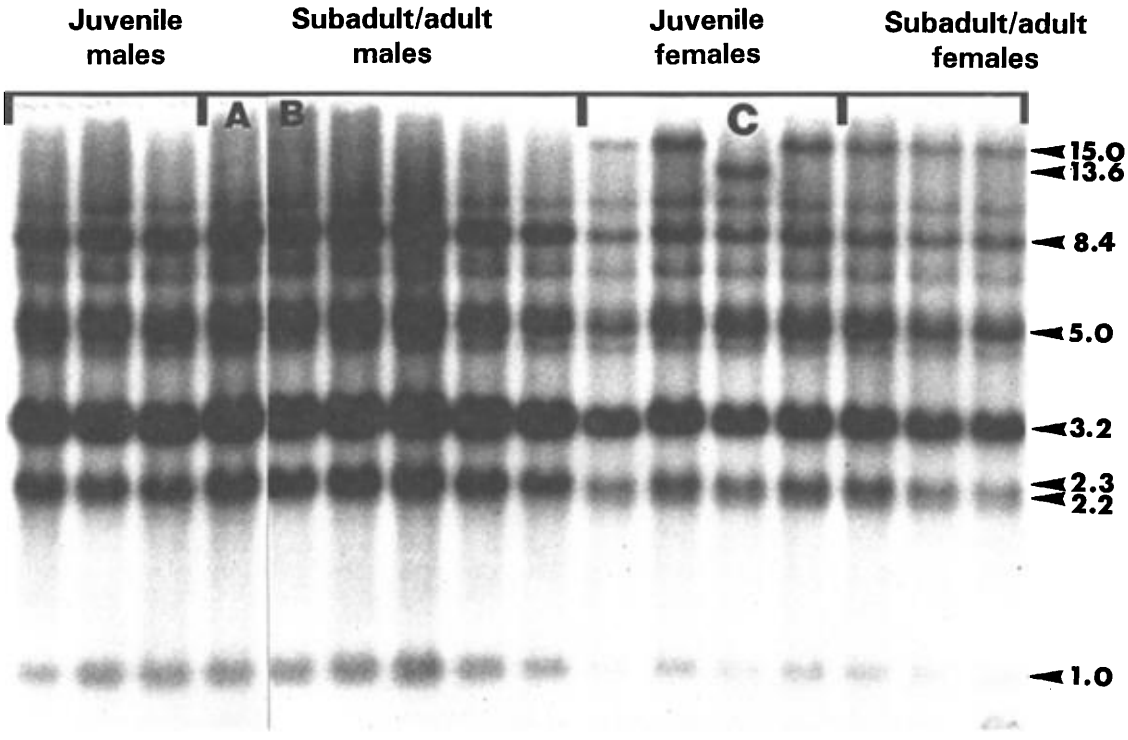

Figure 1. SacI digests of 16 northern spotted owls probed with DZWM1. Lanes A and B are samples from the same bird taken one year apart (1991 and 1992). Lane C shows an uncommon W allele in this study.

to Fuji RX film with two intensifying screens. Overnight exposure was generally sufficient.

RESULTS

We correctly identified the sex of all 45 subadult/adult spotted owls from which sufficient DNA had been collected. Three birds (one male [Fig. 1], two females) were sampled twice, once as fledglings and again as first-year subadults. We also observed three second-year owls (two males, one female; not included in totals above) which, as juveniles, were correctly identified by other researchers who used this same procedure (D. Herter pers. comm., S. Sovern pers. comm.).

An autoradiograph of Southern blot hybridization of DZWM1 on SacI digest of genomic DNA from 16 spotted owls is shown in Fig. 1. The arrows on the right side of the figure indicate the molecular weight size of the bands in kilobases (kb). With SacI digestion, the 15.0 and 13.6 kb bands are found only in female birds, hence are derived from the differentiated region of the W chromosome. The 8.4, 3.2, 2.3 and 1.0 kb bands, which are of double-intensity in male birds, originate from the Z chromosome. The 13.6 kb band found in sample C is an alternate W allele. Though comparatively rare in our Washington samples, it is more common in samples from Oregon (Forsman pers. comm., Halverson pers. obs.). The 5.0 kb band is nonspecific and provides a crude measure of the amount of DNA in each lane.

DISCUSSION

DNA analysis correctly identified the sex of all 62 (45 subadult/adult, three resamples, 14 recaptures) samples of spotted owl blood. This technique can be used by commercial laboratories and has immediate application in studies of spotted owl ecology because it allows rapid sex identification of all juvenile owls and eliminates reliance on recaptures to determine sex (the recapture rate of this sample was only 3.1%; Fleming unpubl. data). Because noninvasive sex determination models based on morphometric features appear capable of accurately sexing most spotted owls (Fleming unpubl. data), we recommend blood sampling only those owls which cannot be reliably sexed by measurement.

Analyses involving other avian species indicate that blood sampling procedures have little effect on individual birds (Stangel 1986, Colwell et al. 1988, Stangel and Lennartz 1988, Ardern et al. 1994). Nonetheless, we attempted to evaluate whether spotted owls were adversely effected by handling and blood sampling. Our resighting rate for adults from which we collected blood samples was 88.9% (40/45). Of the five owls that have not been resighted, two were from areas burned by a catastrophic forest fire in 1994, and there have been no opportunities to resight three owls banded in 1995. It would be difficult to make a similar assessment for juveniles because the resighting rate is very low. However, extensive observations on two adjacent study areas of radio-equipped juveniles from which blood samples were collected indicate that blood sampling does not adversely effect the owls (D. Herter pers. comm., S. Sovern pers. comm.). This leads us to believe that the procedure is safe when properly administered. Because the northern spotted owl is a threatened subspecies, we recommend that all blood samples be collected under required Bird Banding Laboratory special authorization by banders with experience and/or training in blood extraction procedures.

We encountered very few problems using this procedure. Two samples, correctly identified in the laboratory, were initially misidentified when a technician inadvertently trimmed the photographs of the blots too closely. Only 20 of 358 samples (355 birds and three samples that were tested twice), yielded no initial results. Of these, six were misplaced; three contained marginal amounts of blood, but were salvaged when results were obtained on a retest; four contained insufficient blood to analyze; and seven apparently degraded due to heat exposure when transported from the field at temperatures of about 37°C. Heat degradation had not been previously observed in over 50 000 samples from 300 avian species. Because spotted owl blood coagulated rapidly in hot weather (≥29°C) upon exposure to air or to a needle exposed to the sun, we often placed blood directly into the cryovial from the syringe. This occasionally necessitated additional laboratory time to eliminate "extra" sample. The problem was corrected when we later used a heparinized capillary tube to collect blood.

Sex-linked polymorphism exhibited by Southern blot hybridization with DZWM1 is consistently found with other restriction enzymes; the choice of enzyme depends on ease of interpretation and cost. Digestion with alternate enzymes can aid in the analysis of allelic variation such as occurred in sample C. The existence of variant alleles necessitates the development of a data base on a given species before accurate sexing of unknown samples can be guaranteed. We therefore recommend that initial efforts use, if possible, blind samples from known-sex members of pairs to establish species parameters.

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