MORPHOMETRIC, KARYOTYPIC, AND LAPAROSCOPIC TECHNIQUES FOR DETERMINING SEX IN BALD EAGLES

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Accurate sex identification is an important factor in managing and studying both wild and captive animals. Increasing use of captive breeding programs in endangered species management requires an accurate yet safe and convenient way to determine sex in monomorphic species such as the bald eagle (*Haliaeetus leucocephalus*) (Brown and Amadon 1968).

Birds are usually sexed on the basis of plumage or morphological differences, gonadal inspection by laparoscopy, and less frequently by chromosome analysis techniques. In addition, attempts have been made to sex avian species by morphometric measurements (Shugart 1977, Reese and Kadlec 1982, Skeel 1982, Scolaro et al. 1983). Helander (1981) sexed nestling white-tailed sea eagles (*H. albicilla*) using external characteristics. A. R. Harmata and D. W. Stahlecker (unpubl. data) separated bald eagles into two groups based on measurement data. Bortolotti (1984) used measurements from museum skins to devise a predictive model for determining sex in bald eagles. All of these studies based their classification on birds of unknown sex.

Chromosome analysis has had some limited use as a sexing tool in avian species (Hungerford et al. 1966, Mengden and Stock 1979) but is still not a common and widespread practice. Genomes of many Falconiformes have been identified (DeBoer 1976) including bald eagles (Au et al. 1975), making possible short-term blood culturing and karyotyping a means of sexing bald eagles. Surgical sexing techniques on birds were described as early as 1953 by Baily (1953), and present improvements in laparoscopic equipment and techniques (Bush 1980) make this method even simpler and safer. A detailed technique has not been described for bald eagles.

The goals of this study were to: (1) determine an accurate, non-invasive technique of sexing fully grown bald eagles; (2) test the efficiency and practicality of karyotyping as a sexing tool; and (3) refine and evaluate surgical sexing procedures.

METHODS

Forty-one bald eagles admitted for rehabilitation at the Raptor Research and Rehabilitation Program (RRRP), University of Minnesota, and 10 bald eagles from the Eagle Propagation Project (EPP), Milstadt, Illinois, were used. The RRRP eagles were anesthetized with 10–15 mg of ketamine hydrochloride (Bristol) and 2.0–2.5 mg of xylazine (Haver-Lockhart) (Redig 1982) to determine sex by laparoscopy and facilitate the taking of morphometric measurements (detailed below). Sexes of the EPP eagles had been previously determined by laparoscopy. Morphometric measurements were made on them without any anesthesia. A cloth metric tape was used to make measurements of feathers to the nearest 1 mm. Other measurements were made with calipers to the nearest 0.1 mm.

The following measurements were made on each of the birds after the method of Baldwin et al. (1931): (1) body weight (WT) to the nearest 5 g; (2) head length (HL); (3) culmen length (CL) (Fig. 1); (4) culmen length with cere (CWC) (Fig. 1); (5) thickness of midshaft tarsus frontal (MTLF); (6) thickness of midshaft tarsus lateral (MTLL); and (7) tail length (TL).

The following measurements are modifications of those described by Baldwin et al. (1931): (1) forearm length (FAL), measured in a straight line along the leading edge of the patagium of the extended wing from the proximal end of the humerus to the distal end of the radius; (2) head width (HW), width of the skull at the level of the ear openings; (3) beak depth (BD), thickness of the beak from the dorsal aspect of the maxilla adjacent to the cere, to the ventralmost portion of the mandible (Fig. 1); (4) beak width (BW), width of the ventral aspect of the maxilla adjacent to the distal edge of the cere; (5) narrowest tarsal thickness lateral (NTLL), narrowest lateral thickness of the tarsometatarsus (This

Fig. 1. Schematic drawing of bald eagle head showing the following measurements: (A) culmen length with cere (CWC); (B) beak depth (BD); and (C) culmen length (CL).

point usually occurred 2–3 cm proximal to the distal end of the tarsometatarsus, near the junction of the feathers and tarsal scutes. The calipers were closed until they met slight resistance. Several attempts were needed to make this measurement.); and (6) narrowest tarsal thickness frontal (NTLF), similar to (5) except measured anteriorly–posteriorly.

Repeatability of measurements and descriptive quality of morphometric definitions were tested in two trials using three and four volunteers, respectively. Participants were selected who were familiar with avian anatomy but had no experience with our technique. Results were tested using ANOVA.

Chromosome karyotyping on cultured peripheral lymphocytes followed standard techniques used for mammalian blood (Lin et al. 1976). Separated lymphocytes were cultured in RPMI 1640 media (Gibco) containing streptomycin (Eli Lilly & Co.) at 100 μg/ml and penicillin (Parke Davis) at 100 units/ml. They were incubated 72 hours in a 5% CO_2 atmosphere at 37 C. Antibiotic concentrations were doubled for samples from eagles with ongoing bacterial infections. Four mitogens, Phytohemaglutinin-p (Gibco), Pokeweed (Gibco), Lipopolysaccharide (Difco), and Concanavalin-A (Miles) were tested for their ability to stimulate lymphocytes. After fixation with methanol : glacial acetic acid (3:1), a modification of the barium hydroxide method of Sumner (1972) was used to C-band the chromosomes. The presence or absence of the dark staining female W chromosome (Au et al. 1975) was used to determine the sex.

Laparoscopy was performed with modifications on the method of Redig (1979) using an otoscope (Welch-Alyn, Skaneateles Falls, N.Y.) with a 7-mm speculum or a 5-mm fiber optic focuscope (Medical Diagnostics Service, Brandon, Fla.). Anesthetized eagles were restrained in right lateral recumbancy. The left wing was drawn cranially and the left leg extended distally. An area of 4–5 cm^2 cranial to the femoral musculature was plucked of feathers and prepared for aseptic surgery. The landmarks used to locate the surgical site were the junction of the vertebral and sternal components to the last pair of ribs and the cranial edge of the sartorius muscle. A 2-cm incision was made in the skin. After palpating the last intercostal space, which was deep to the cranial edge of the sartorius muscle, we made a stab incision through the intercostal musculature. A 4.7-mm ($\frac{3}{16}$-inch) Steinman pin with a trocar tip was used to puncture the abdominal air sac, and the speculum of the otoscope was inserted. The adrenal gland and the anterior lobe of the kidney were used as the internal landmarks to aid in locating gonads. Incisions were closed by suturing the cranial edge of the sartorius to the intercostal musculature, and the skin was closed with a continuous pattern of 3-0 surgical gut. In some cases only the skin was sutured.

Discriminant function analysis (DFA) utilizing the jackknife option (Jennrich and Sampson 1981) was performed on the data. A *t* test (Zar 1974) was used to determine if mean measurements differed between age-classes.

RESULTS

Prescribed doses of anesthesia provided a working time of approximately 30 minutes, with an additional 4 hours required for full recovery. Laparoscopy took approximately 5 minutes, and approximately 20 minutes were required to make all of the measurements.

Twelve measurements were taken on the birds, and only BD showed no overlap in values between the sexes (Fig. 2). HL, NTF, and NTLL had little overlap between the outlying extremes of the sexes but none in the 25–75 percentile (Fig. 2). The stepwise DFA yielded two models. Model 1 involved BD, NTLF, HL, and NTLL, which correctly classified 97.7% (N = 38) of our cases when compared to laparoscopy and karyotyping. Model 2 used only two measurements, BD and NTLF, and correctly classified 97.7% (N = 44) of our cases. Although both models offered the same degree of confidence in classifying sexes, the four-variable

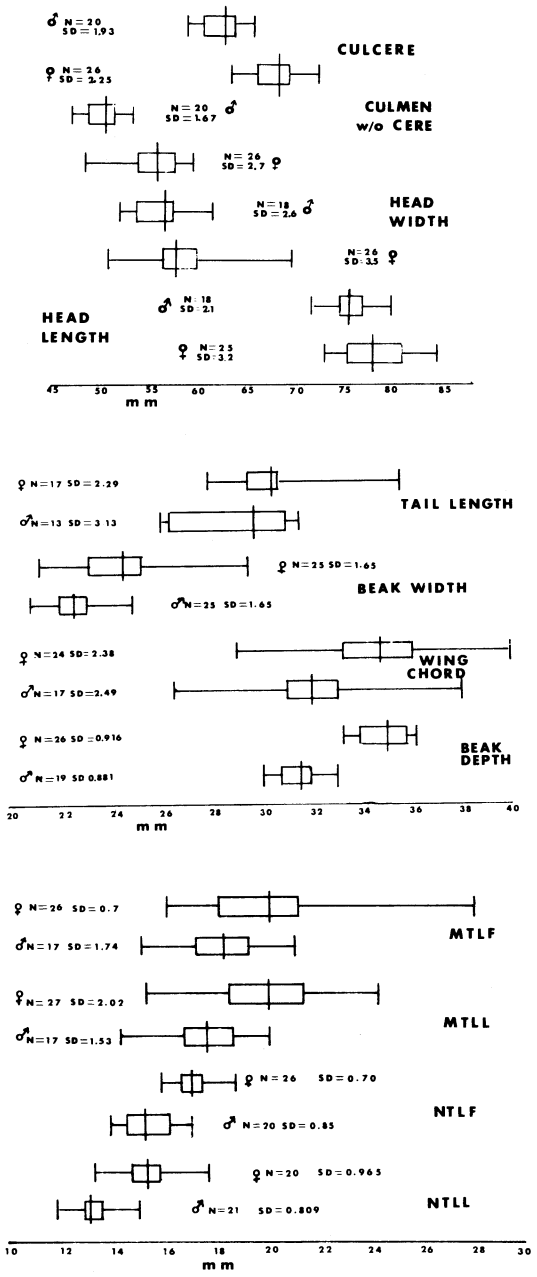

Fig. 2. Range of measurements taken on male and female bald eagles. Boxed area represents 0.25–0.75 percentile, vertical line is the mean, and horizontal line represents the tail 0.25 percentiles. MTLF—thickness of midshaft tarsus frontal; MTLL—thickness of midshaft tarsus lateral; NTLL—narrowest tarsal thickness lateral; NTLF—narrowest tarsal thickness frontal.

Table 1. Discriminate function coefficients for two morphometric measuring models for sexing bald eagles.

Model 1 (N = 38)		Model 2 (N = 44)	
Variable	Coefficient	Variable	Coefficient
BD[a]	1.0118	BD	0.92361
HL	−0.18206	NTLF	0.61678
NTLL	0.43907	Constant	40.66435
NTLF	0.45319		
Constant	33.35617		

[a] BD—beak depth; HL—head length; NTLL—narrowest tarsal thickness lateral; NTLF—narrowest tarsal thickness frontal.

model (1) showed an 11.4% greater separation of group means. First- and 2nd-year females (N = 8) showed no difference from adults (N = 10) in Model 1 measurements ($P > 0.05$), and 1st- and 2nd-year males (N = 7) were different ($P < 0.05$) from adult males (N = 10).

Use of feather measurements to discriminate between sexes could cause erroneous results due to size correlation with age-class. In 1st-year males with complete tails, mean length was 33.3 cm (N = 3). In males of subsequent age-classes mean was 28.0 cm (N = 12). Females also showed dissimilarity, with 1st-year means of 34.1 cm (N = 3) and subsequent age-class means of 29.9 cm (N = 14).

To determine sex of an unknown bird, we multiplied each measurement in a model by its respective coefficient (Table 1), and the products were summed. If the total was less than the model's constant, the bird was a male; totals greater than the constant indicated females; sums equal to the constant were of unknown sex.

A test of the repeatability of the BD, HL, and NTLL (three biologists measuring three birds) showed no difference ($P > 0.10$). Of 28 cases where surgical sexing was attempted, 1 (3.5%) was not sexed owing to equipment failures, and 2 (7.1%) were determined to be males by default. Forty-one samples from 32 birds were tested in the karyotyping procedures. These yielded 20 successfully C-banded preparations, of which 19 (95%) were correctly identified. Concanavalin-A was the most effective mitogen.

DISCUSSION

The goal of the morphometric measurement procedure was to derive simple, repeatable measurements that clearly separated sexes. We found Model 2 preferable because the HL mea-

surement was difficult to make and could be stressful to unanesthetized birds. Bortolotti (1984) found TL to differ significantly between adults and immatures, which our data support. Although 1st- and 2nd-year males differed significantly from adult males in the Model 1 measurements, means for the younger birds were smaller, so they would not be confused with females.

With the laparoscopic technique, immature birds, especially in their 1st and 2nd year (2nd-year age-classification known due to length of stay in captivity), were the most difficult to sex because of undeveloped gonads. Any evidence of nondifferentiated gonadal tissue was considered indicative of females, and no evidence of gonadal tissue was indicative of males ("males by default"). Two birds were called males by default, and their sex was subsequently confirmed by karyotyping. Deposits of visceral fat, common in captive birds, can complicate locating and discerning sex organs.

Although laparoscopy was found to be a safe form of determining sex, post-operative infection and anesthetic complications are potential risks. The use of aseptic techniques (sterilization of instruments, disinfection of surgical site) prevented the occurrence of infection in this study and should be used. Anesthetic complications did not occur. The combination of ketamine hydrochloride and xylazine has proven to be an extremely reliable and effective technique for bald eagles and most other species of raptors (Redig 1982). No change in behavior was noted following these procedures.

Karyotyping was less reliable than other techniques because growing lymphocytes for chromosome analysis was difficult. Contamination of samples, chronic infections, or birds undergoing antibiotic therapy all seemed to inhibit lymphocyte growth. The poor percentage of cases karyotyped is partly reflective of initial experimentation required to determine the most effective mitogen. Incorrect sex determination can be due to poor results in the C-banding procedure. When the chromosome spread stains too lightly, the W chromosome (if present) does not show up and the cell can be interpreted as a male.

Each technique tested shows promise for sexing bald eagles under different circumstances. Nestling bald eagles that would not be sexed accurately by our measurements or by laparoscopy could be sexed by karyotyping. Laparoscopy affords investigators an opportunity to observe the functional state of the gonads as well as accurately determine sex. Factors that could hamper normal gamete production and development can be detected and such birds culled from a captive breeding flock. Determining sex of eagles in the field poses problems for both the karyotyping and laparoscopy techniques. Individuals surgically sexed by the method described should be monitored for 3–4 days prior to release to ensure that no complications develop. Furthermore, aseptic conditions are not usually available under field situations. Blood drawn for karyotyping needs to be processed within 48 hours. A viable alternative exists in culturing feather pulp (Van Tuinen and Valentine 1982), which allows longer storage time and is non-invasive. We believe morphometric measurements are the most suitable for field situations due to the ease, safety, and accuracy of the technique. Model 1 and 2 measurements can be performed in less than 10 minutes even on unanesthetized birds. Because most eagles used in the study were from the north-central United States and Canada, application of this technique on eagles throughout their range may not provide the same degree of correct classification with our models.

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LITERATURE CITED

AU, W., N. S. FECHHEIMER, AND S. SOUKUP. 1975. Identification of the sex chromosomes in the bald eagle. Can. J. Genet. Cytol. 17:187–191.

BAILY, R. E. 1953. Surgery for sexing and observing gonad condition in birds. Auk 70:497–499.

BALDWIN, S. P., H. C. OBERHOLSER, AND L. G. WORLEY. 1931. Measurements of birds. Cleveland Mus. of Nat. Hist. Sci. Publ., Vol. 2. 165pp.

BORTOLOTTI, G. R. 1984. Sexual size dimorphism and age-related size variation in bald eagles. J. Wildl. Manage. 48:72–81.

BROWN, L. H., AND D. AMADON. 1968. Eagles, hawks and falcons of the world. McGraw-Hill Book Co., New York, N.Y. 945pp.

BUSH, M. 1980. Diagnostic avian laparoscopy. Pages 97–100 *in* J. E. Cooper and A. G. Greenwood, eds. Proc. Int. Symp. on diseases of birds of prey. Chiron Publ., Keignley, West Yorkshire, U.K.

DEBOER, L. E. M. 1976. The somatic chromosome

complements of 16 species of Falconiformes (Aves) and the karyological relationships of the order. Genetika 46:77–113.

HELANDER, B. 1981. Nestling measurements and weights from two white-tailed eagle populations in Sweden. Bird Study 28:235–241.

HUNGERFORD, D. A., R. L. SNYDER, AND J. A. GRISWOLD. 1966. Chromosome analysis and sex identification in the management and conservation of birds. J. Wildl. Manage. 30:707–712.

JENNRICH, R., AND P. SAMPSON. 1981. Stepwise discriminate analysis. Pages 519–537 *in* W. J. Dixon, ed. BMDP statistical software. Univ. of California Press, Berkeley.

LIN, C. C., D. R. NEWTON, W. K. SMINK, AND R. B. CHURCH. 1976. A rapid and simple method for the isolation and culture of leukocytes for chromosome analysis in domestic animals. Can. J. Anim. Sci. 56:27–31.

MENGDEN, G. A., AND A. D. STOCK. 1979. A preliminary report on the application of current cytological techniques to sexing birds. Int. Zoo Yearb. 16:138–141.

REDIG, P. T. 1979. Surgical approach for sexing birds of prey. Pages 252–253 *in* M. E. Fowler, ed. Zoo and wild animal medicine. W. B. Saunders Co., Philadelphia, Pa.

———. 1982. Overview of avian anesthesia. Proc. Assoc. of Avian Veterinarians 1982:127–139.

REESE, K. P., AND J. A. KADLEC. 1982. Determining the sex of black billed magpies by external measurements. J. Field Ornithol. 53:417–418.

SCOLARO, J. A., M. A. HALL, AND I. M. XIMENEZ. 1983. The magellanic penguin (*Spheniscus magellanicus*): sexing adults by discriminant analysis of morphometric characters. Auk 100:221–224.

SHUGART, G. W. 1977. A method for externally sexing gulls. Bird Banding 48:118–121.

SKEEL, M. A. 1982. Sex determination of adult whimbrels. J. Field Ornithol. 53:414–416.

SUMNER, A. T. 1972. A simple technique for demonstrating centrometric heterochromatin. Exp. Cell Res. 75:304–306.

VAN TUINEN, P., AND M. VALENTINE. 1982. A noninvasive technique of avian tissue culture (feather pulp) for banded chromosome preparations. Mammal. Chrom. Newsl. 23:182–286.

ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall Inc., Englewood Cliffs, N.J. 622pp.

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COMPENSATORY REPRODUCTION THROUGH RENESTING IN WILLOW PTARMIGAN

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Abstract: Willow ptarmigan (*Lagopus lagopus lagopus*) were censused in late summer for 4 years and broods classified as first, renest, or null (empty) broods. From brood frequencies and clutch size data, estimates were made of egg production, hatching success, survival of young, and contribution to production of each class. Loss of first clutches (N = 231) averaged 27%, and renesting compensated for 43% of the eggs lost. Clutch loss and survival of chicks were similar for both classes. Adults composed 18% (N = 462), first nest chicks 70% (N = 1,792), and renest chicks 12% (N = 299) of the late summer population. Renest chicks compensated for an estimated 45% of the first nest chicks lost due to first clutch destruction. Production from renests was close to the maximum possible during this period of high production and rapid population increase.

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Many single-brooded species respond to nest destruction by laying repeat or renest clutches. Renesting has been documented for most members of the Tetraonidae (Angelstam 1979, Parker 1981, Storaas et al. 1982), the ring-necked pheasant (*Phasianus colchicus*) (Seubert 1952, Warnock and Joselyn 1964, Gates 1966, Dumke and Pils 1979), and probably occurs among all galliforms. Errington (1942) derived an equation from which the contribution of renestings to brood production could be calculated, but for a hypothetical species. In this study, estimates are made of the success of the first and renest clutches, survival of young from first and renest broods, and the contribution of both classes to production, based on quantitative information about eggs and young from intitial and repeat layings.

METHODS

Fieldwork was conducted from 1978–81 on Karlsøy Island (70°00'N, 19°55'E), Troms Coun-