Sex Determination in Psittaciformes

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Key Words: Sex Determination, PCR, Endoscopy, Psittacine birds

Many avian species are considered sexually monomorphic. While most of these species probably have subtle physical characteristics that could be used to distinguish between the sexes, these differences are not readily visible. Detailed studies to determine suitable differences between the sexes have not been

reported for most common companion birds species. Techniques that have been described for sex determination in "monomorphic" species include examination of the cloaca, measurement of steroid levels in plasma or excrement, chromosomal analysis, laparoscopy, and use of sex-specific nucleic acid primers and probes (DNA amplification and detection).[1]

Male Galliformes, Anseriformes, and ratites have a phallus which can be visualized by examining (chicks) or palpating (adults) the cranial wall of the cloaca. Male Columbiformes and Passeriformes have a prominent papillae of the ductus deferens that can be visualized on the cranial wall of the cloaca.[1] Measurement of the estrogen and androgen levels in plasma or excrement has been used to determine the sex of some birds. However, the quantity of steroid hormones produced varies with the age and sexual activity of a bird, which complicates interpretation of the estrogen/androgen ratio.[1]

In birds, the female is heterogametic (Z and W chromosomes) and the male is homogametic (two Z chromosomes). Cultured cells from a bird (most commonly cells derived from a developing feather) can be used as a source for chromosome isolation and karyotyping. Successful chromosome analysis by an experienced cytogeneticist should be absolute, within scientific definable limits, and can provide information that cannot be ascertained from any other technique. The primary disadvantage to chromosome analysis is the difficulty in obtaining viable cells for culture and a slow response time.[1]

Laparoscopic identification of the gonads by an experienced endoscopist is often used as the "gold standard" for sex determination, although it could be argued that chromosome analysis should be the gold standard. Laparoscopic sexing is particular advantageous because it provides immediate results and one can evaluate physical characteristics of the reproductive tract (as well as other organ systems). Gonadal tissue from a mature bird is easily visualized as compared to that of a juvenile but an experienced endoscopist can determine the sex of chicks. The primary disadvantages of laparoscopy are the necessity of anesthesia and invasion of the coelomic cavity.

An increased number of birds are being sexed using DNA amplification techniques that differentiate between unique nucleic acid sequences found in the W and Z chromosomes. While these techniques offer the advantage of a non-invasive sexing method and do not require anesthesia, the only information provided is the sex of the bird. Information about the morphologic condition of the reproductive tract, the gross condition of other organs and the character of the chromosomes are not obtained. Additionally, these tests are subject to errors caused by contamination that is inherent with any DNA amplification and detection assay. The greatest potential for contamination occurs when blood samples are collected from a toenail or when feathers are used in lieu of blood. Blood collection by toenail clipping and/or submission of feathers are being advertised as methods for aviculturist to obtain samples to submit for sex determination. Washing the nail prior to sample collection, as is suggested, does not reduce the potential for contamination. For example, if the target segment of nucleic acid from the W chromosome, which is present in every cell in the heterozygous female bird,

were to contaminate the feather of a male and the male's feather was used for sex identification, the male bird could be incorrectly identified as a female. Contaminating a male's sample with nucleic acid from another male would not effect the results.

Because of the increased use of DNA amplification techniques for sex determination, a study was performed to compare the reliability of a DNA amplification test[a] (through use of blood and feathers) to direct visualization of the gonads by endoscopy or at necropsy. Additionally, data was collected on the gross appearance of the reproductive tract and other internal organs to determine how many reproductive and other problems would have been missed if sex had been determined using DNA amplification to the exclusion of endoscopy.

The target study population is 1000 mixed species of Psittaciformes. To date, approximately 400 birds have been evaluated including cockatoos, macaws, conures, cockatiels, Amazon parrots, African grey parrots, eclectus parrots, *Poicephalus spp*, caiques, lories, lovebirds and pelicans. Samples submitted for DNA amplification include whole blood collected by venipuncture and placed in heparin or EDTA and at least 4 fully differentiated contour feathers.

In this study, results obtained using blood for DNA amplification agreed with the sex as determined by visualization of the gonads in all but 3 samples; one of these birds had a grossly abnormal gonad, with no other identifiable reproductive tissues. As expected, approximately 4% of birds that were identified as males based on direct visualization of the testicle and by DNA amplification of blood were incorrectly identified as females using feathers for DNA amplification. The assay successfully differentiated between male and female of all the Psittaciformes in this study except for lories. The assay reliably identified female lories but detection of target sequence for males requires additional processing. Approximately 15% of the birds in this study had lesions of the reproductive tract or other organ systems that could at least affect reproductive performance.

Based on the results of this study, it is the opinion of the authors that endoscopic evaluation of the reproductive tract and other internal organs by an experienced endoscopist is the best method for sex identification in birds that are being used for breeding purposes. Laparoscopy in conjunction with chromosome analysis would provide information about the condition of the reproductive tract and detect some chromosomal defects that could affect reproduction. Blood samples, collected by venipuncture, can be used in well-validated and controlled DNA amplification tests to determine the sex of many psittacine birds, if the convenience of this assay is more important than inherent inaccuracy as a result of sample contamination. The use of feathers for sex identification should be used only when a relatively high inaccuracy rate is acceptable.

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1. Joyner KL. Theriogenology. *In* : Ritchie BW, et al (ed). Avian medicine: principles and application. Lake Worth: Wingers Publishing, 1994: 748-804.