I. Surgical Sexing Methods

A. Historical Comments and Procedures

Direct visual examination of internal avian gonads (male testes or female ovary) is an old and proven sexing method. It is applicable to many types of birds, even when performed under field conditions or as a house call service. The gonads can be seen with the naked eye through a small incision (10-15 mm) made through the skin and abdominal wall of the bird’s left side near the last rib. New and more sophisticated equipment has improved viewing of the gonads through a smaller incision. The otoscope (also used for ear, nose and throat exams) and the laparoscope (a fiberoptic telescope) are currently preferred. The basic surgical approach is still on the bird’s left side near the last rib with minor variation. Safer injectable anesthetic drugs are available, but the need for general anesthesia during surgical sexing is a debatable issue. Many veterinarians prefer only to restrain the bird, thus eliminating the risks of anesthetic complications. Either procedure is well-tolerated by many types of birds and complications are relatively infrequent. However, with any surgical procedure complications can result in an unexpected death of a bird. Your best assurance for safety is a healthy bird and an experienced avian clinician. The cost of surgical sexing ranges from $15 to $75 per bird as determined by the individual practitioner. Some even offer house call service.

B. Advantages

The major advantages (see chart for comparisons) are immediate results without delay for lab analysis and the potential for sexing immature and/or inactive birds. A physical exam of the bird is also recommended and should accompany surgery. Reproductive condition may be approximated by comparing the relative size of a bird’s gonads. This is very dramatic when looking at the ovary of a hen just before laying occurs. Physical abnormalities of the bird’s reproductive tract may also be identified, as well as several disease problems with other internal organs.

C. Disadvantages

The major disadvantages (see chart) concerns trauma and stress from handling the bird, also the risks of surgical complications and ultimately the death of a bird. Mortality rates are variable (1-10% +) and unpredictable. An experienced avian clinician should be sought although one may not be available in your area. Obese birds, diseased birds or those with immature and undeveloped gonads may not be surgically sexable. The long-term effects of surgical sexing upon future reproduction are not fully known; detrimental effects have not yet been documented.

II. Hormonal Sexing Method

A. Historical Comments and Procedures

Avian gonads have two major functions: 1) to produce gametes (eggs or sperm), and 2) to produce hormones (sex steroids) which act throughout the body like messengers to stimulate and regulate sexually related growth and behaviors necessary for reproduction. Gonadal functions in humans and domesticated animals have been studied for many years by measuring the level of hormones in blood or tissues or less frequently in the animal’s urine. Blood or tissue samples were not easily collected from exotic birds and separate urine collection is not possible in birds. Therefore, research began at the San Diego Zoo to investigate a non-invasive fecal steroid analysis method which measured the level of metabolized hormones in the mixed feces and urate droppings of birds. Total excretory estrogens (E) and testosterone (T) hormones were measured and evaluated as an E/T ratio. Both types of hormones are produced by the gonads of each sex, but in different proportions. Functionally, mature female parrots produce more E than T and are indicated by a high E/T ratio (greater than 2.0). Sexually mature male parrots produce more T than E and are indicated by a low E/T ratio (less than 0.6). Immature or sexually inactive birds are frequently indicated by an E/T ratio approaching 1.0. Due to low daily hormone levels produced by inactive birds, they may not be consistently sexable by current hormone methods. Validation of hormonal sexing has involved extensive comparison of E/T ratio data from a wide variety of known-sex birds to determine the range of acceptable values for unsexed males and females, based on the specific analytic techniques described.

Laboratory analysis for micro quantities of excretory hormones involves a radioisotope technique, expensive equipment, and a trained technician. It has just recently become available to the general public through a private commercial laboratory specializing in hormonal sexing of parrots. The cost for hormonal sexing ranges from $50 to $70 per bird, depending upon the number of birds to be sexed.
B. Advantages

The major advantage concerns non-invasive collection of fecal droppings. Birds need not be handled, stressed or moved from their present aviaries if an identified sample can be collected. A perfect record of no deaths or serious injuries means that hormonal sexing can be safely applied to rare and endangered or very valuable specimens as well as common species. Frozen samples can be easily shipped from virtually anywhere in the United States. Reproductive condition of the birds can be estimated by their relative hormone levels; active birds with functional gonads produce more steroids than inactive birds, regardless of age, which produce low hormone levels. Sexing results can be available within 2-3 weeks of sample shipment.

C. Disadvantages

The major disadvantages concern low daily hormone production from immature parrots, less than three years of age, or seasonally inactive birds which may not be consistently sexable by current hormone analysis techniques. Repeat sampling and analysis is recommended during the next breeding season for these cases. Disease and/or chronic stress can also interfere with normal gonad function and prevent hormonal sexing in affected birds.

III. Genetic Sexing Methods

A. Historical Comments and Procedures

All plant and animal cells contain in their nucleus the basic units of heredity (genes). During one stage of cell growth and replication (mitosis) these minute nuclear structures consolidate in a linear arrangement and become visible under the microscope as paired bodies called chromosomes. Each species has a characteristic number of chromosomes and a single pair of sex chromosomes. Laboratory techniques have been developed for growing white blood cells which maximize the visualization of chromosomes. Avian sex chromosomes can then be examined and classified by the "ZW" method which describes their sexually distinct physical appearance, the Z-chromosomes are always larger than the W-chromosomes. Male birds are sexed by the observation of a matched pair of ZZ-chromosomes and females are sexed by the observation of a mixed pair of ZW-chromosomes. All cells in a bird's body contain these chromosomes, although they are identified more easily in rapidly growing cells, i.e., white blood cells or the pulp tissue in a growing pin feather. Blood culture is often preferred.
because a larger number of cells can be found in the mitotic stage where chromosomes are visible. Chromosome technology has principally evolved from human and mammalian studies; avian chromosome techniques are relatively new and require more research before they are routinely applied. The cost of human chromosome analysis is approximately $150. Since avian chromosome techniques are not routine their cost would be determined by the laboratory involved, and results available within 1-2 weeks.

B. Advantages

The major advantage for chromosomal sexing is that it can be applied to birds of any age or condition, if mitotic cells can be isolated and examined. It also truly represents the genetic sex of a bird, irrespective of behavioral or functional characteristics. Chromosomal abnormalities, although not fully classified in exotic avian species, can be an indication of inherited reproductive problems.

C. Disadvantages

The major disadvantages of genetic sexing concern technical difficulties in culturing and isolating complete bird chromosome patterns and lack of available labs working with avian samples. Handling and restraint are necessary in the collection of blood or pin feather samples which can result in stress and trauma to the bird. The transport of blood samples to a lab may also be difficult for they must be protected from temperature extremes and begin analysis procedures within 24 hours after collection.

CONCLUSIONS

Reliable, repeatable and documented techniques are the key for sex determination in monomorphic birds. The exchange of information and methods between more than one lab or clinic is vital. This paper points out only three scientific techniques. The best method really depends upon the specific needs of each aviculturist. Other sexing methods are available which may not have been as thoroughly tested, some will stimulate further studies and some will remain unsubstantiated. Should you want further information about these methods, I urge you to attend the upcoming 7th Annual AFA Convention, in San Diego, August 5-9, 1981. A portion of the Veterinary Seminar Program is planned with discussions from experts in each method.

The importance of sexing your birds by any accurate method cannot be understated. Having a "pair" of birds only means that two birds are involved. The fundamental requirement of any captive breeding program is to have true pairs; only then can the problems of compatibility, proper environment, and adequate nutrition be evaluated and the potential for reproductive success be maximized.

REFERENCES


### SUMMARY OF PROVEN BIRD SEXING TECHNIQUES

<table>
<thead>
<tr>
<th>METHOD</th>
<th>PROCEDURE</th>
<th>SOURCE</th>
<th>COST/BIRD</th>
<th>SUCCESS RATE</th>
<th>MAJOR ADVANTAGES</th>
<th>MAJOR DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical Sexing</td>
<td>Direct Gonad Examination through small incision in bird’s left side near last rib. Results: Testes in ♂ Ovary in ♀</td>
<td>Veterinary clinics or house call service¹</td>
<td>$15-75</td>
<td>97% + by a skilled clinician</td>
<td>Immediate results Available for most species. Immature birds may be sexable</td>
<td>Mortality rate 1-10%. Unpredictable trauma and stress from handling bird. Few experienced bird practitioners</td>
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<td>Laparotomy</td>
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<td>Laparoscopy</td>
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<td>Endoscopy</td>
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<tr>
<td>Hormonal Sexing</td>
<td>Estimation of Gonadal Function by microanalysis of hormones in bird’s droppings² Results: Low hormone ratio in adult ♂ High hormone ratio in adult ♀</td>
<td>Fecal samples shipped to a commercial lab for analysis¹</td>
<td>$50-70</td>
<td>98% + for mature birds in breeding season</td>
<td>Perfect safety record—no deaths. Available for mature psittacines. Frozen samples easily shipped to lab</td>
<td>Parrots younger than 3 years may not be sexable. Birds not in breeding condition may not be sexable. Quality lab costs are expensive</td>
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<tr>
<td>Fecal steroid analysis</td>
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<tr>
<td>Genetic Sexing</td>
<td>True Genetic Sex determined by isolating sex chromosomes from blood or pin feather cells Results: ZZ chromosomes in ♂ ZW chromosomes in ♀</td>
<td>Research techniques not available to</td>
<td>Undefined</td>
<td>90% + by skilled lab technician</td>
<td>Birds of any age or condition may be sexable</td>
<td>Trauma and stress from handling bird. Technical difficulties can prevent chromosome isolation. Perishable blood sample shipment may be difficult</td>
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<td>Chromosome analysis</td>
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¹Consult local Veterinary Medical Association for referrals to avian practitioners.

²Fecal steroid analysis of estrogens (E) and testosterone (T) hormones are evaluated as an E/T ratio. Mature males have E/T ratios less than 0.6 and mature females have E/T ratios greater than 2.0.

³The Center for Reproductive Studies, Michael M. Mulier, Ph.D., Director, 7079-E Mission Gorge Road, San Diego, CA 92120 now offers hormonal sexing to the general public. This referral does not constitute an endorsement of any private business by the Zoological Society of San Diego.
Nairobi Village Lagoon at San Diego Wild Animal Park.

These photos illustrate two of the many beautiful and interesting sights you will see on the AFA Convention tours. It's a little more difficult for us to show you the beautiful summer climate. Come to the AFA Convention and find out for yourself!

Queensland Koala, Phascolarctos cinereus adustus, "Matilda and Koobor", at the San Diego Zoo.