Chapter 51

SEX DETERMINATION TECHNIQUES

SUSAN L. CLUBB

Accurate sex determination techniques have allowed aviculturists in recent years greater opportunities for successful propagation of monomorphic avian species. The first requirement for a successful captive breeding program is a true pair. Many species of psittacine birds and other species important in aviculture show no obvious sexual dimorphism.

SEXUAL DIMORPHISM

Eclectus Parrots (*Eclectus* sp.) are an example of striking sexual dimorphism in which the male bird is a brilliant emerald green and the female bird is deep red and purple. For many years the male and female were considered to be different species, leading to obvious difficulties in captive propagation. Unfortunately sexual dimorphism is not obvious in many species of psittacines.

There are a few characteristics that are helpful in visually sexing monomorphic birds. These are, however, only indications of sex and cannot be depended upon for accurate sexing of pairs for breeding. Size and shape of the beak and head often give an indication of sex in psittacines. The male bird usually has a larger head and a broader, heavier beak than the female. Male cockatoos are usually significantly larger than females.

Behavioral differences are helpful in sexing some species. The male will often exhibit a courting song and dance while the female observes. This is more obvious in passerines than psittacines and is the most practical means of sexing Society Finches. Likewise, a parrot that spends an excessive amount of time rooting around on the cage floor is likely to be a female that is interested in nest building. Male birds of many species tend to be more aggressive and less fearful than female birds. It is also apparent that the female bird is more likely to bite and will protest more loudly when restrained.

Copulation is observed frequently in improperly paired birds (two males or two females) and is no indication of a true pair. Homosexual pairs are uncommon if birds are allowed to choose a mate by placing birds in a group. Groups must be watched carefully for the formation of pair bonds. If one pair decides to breed, they may become very aggressive toward other birds in the group. Several nest boxes should be provided to reduce fighting over nesting sites.

Palpation of the pelvic bones is a commonly reported sexing method. The pelvic bones are located slightly cranial to the vent and can be palpated with the bird in dorsal recumbency in the palm of the hand with the head restrained. Some people prefer to palpate the bird while it is in a standing position and grasping a perch or finger. In the adult female the pelvic bones are reportedly farther apart than the corresponding distance in the adult male. In addition, the pelvic bones of the female are supposedly more pointed and in the male they are more rounded and directed medially. When used on birds of known sex, however, this test is highly inaccurate.

Sexual dimorphism in psittacines is summarized in Table 51–1. Species that are uncommon in aviculture have been omitted. Australian and Asian species are often dimorphic, whereas South American and African species are usually monomorphic. Birds that inhabit arid climates show a higher incidence of sexual dimorphism than jungle species. This may indicate more dependence on sight identification among species in arid climates. ^{9, 13}

With intensive observation of a species, slight differences may be observed which will be helpful in visual sex determination. For example, African Grey Parrots (*Psittacus erithacus erithacus*) can be sexed with some accuracy by the shade of grey of the plumage. The female has a pale powder grey breast and back, whereas the male is a darker charcoal grey.

Table 51-1. SEXUAL DIMORPHISM IN PSITTACINES*9, 11, 13

PARROTS OF AUSTRALIAN AND PACIFIC DISTRIBUTION

LORHDAE—Most species are monomorphic. The head is usually larger in male birds.

Dusky Lory—Genus Pseudeos. Monomorphic.

Black Lory, Duvienbode—Genus Chalcopsitta. Monomorphic.

Red Lories—Genus Eos. Monomorphic

Chattering Lory—Genus Lorius. Monomorphic.

Rainbow Lory—Genus Trichoglossus. Meyer's Lorikeet (T. flavoviridis meyeri)—the male has a larger and brighter yellow ear patch than the female.

Stella Lory—Genus Charmosyna. Stella Lory is dimorphic. The female has a yellow patch on the rump and lower back.

Blue Lories—Genus Vini. Monomorphic.

CACATUIDAE

CACATUINAE

Palm Cockatoo—Genus Probosciger. Monomorphic but male is usually larger and has a larger beak. The size difference varies geographically and according to subspecies.

Black Cockatoo—Genus Calyptorhynchus. Sexual dimorphism is striking in some species and barely evident in others. In the Banksian Cockatoo (C. magnificus), the male plumage is black except for bands of red in the tail whereas the female plumage is dotted and barred with yellow-orange.

Gang Gang Cockatoo—Genus Callocephalon. The male is slate grey with a red head and crest. The female has a

grey head and crest and plumage that is barred with greyish-white. White and Pink Cockatoos—Genus Cacatua and Eolophus. Adult birds except for the Bare-eyed Cockatoo (C sanguinea sanguinea) can be sexed by eye color. The female has a red iris, whereas the iris of the male is dark brown to black. A bright light may be needed to determine eye color in some species. The female of most species is smaller than the male. Red-eyed males and dark-eyed adult females have been reported but are rare. The image brown in immature birds of both sexes.

NYMPHICINAE

Cockatiel—Genus Nymphicus. In the wild type (grey) the sexes are easily distinguished by the bright yellow markings in the male which are absent in the female. Cinnamon, white, or albino cockatiels can be sexed by faint diagonal bars on the ventral surface of the primary and secondary flight feathers of the female, which absent in the male. Pied cockatiels are difficult to sex and if heavily pied require surgical sexing. Cinnamou cockatiels are sexed by faint yellow facial coloration in the male. Pearl cockatiel males lose the pearl coloration upon maturity and resemble the normal grey coloration. Only female and immature birds will exhibit the pear coloration. Most birds exhibit mature plumage coloration at six to nine months of age.

PSITTACIDAE

Budgerigar—Genus Melopsittacus. In the normal green variety, the cere of the male is blue, whereas the cere of the **PSITTACINAE** female is pinkish-brown. This is not dependable in hybrid color variations, e.g., yellow, blue, or white birds.

Rosellas—Genus Platycercus. The male of most rosella species is slightly brighter than the female or immature. Female and young of several species have a row of white spots on the ventral surface of seven or eight primary and secondary flight feathers. These are lost by the male upon reaching sexual maturity. The wing spots are retained in adult female Yellow Rosella (P. flaveolus), Golden-mantled Rosella (P. eximius), Mealy Rosella (P adscitus), and Stanley Rosella (P. icterotis).

Red-rumped Parakeet—Genus Psephotus. Most species in the genus exhibit sexual dimorphism. The Red-rumped Parakeet exhibits pronounced sexual dimorphism. The male has a red patch on the rump. The female is more

drab. Other species are uncommon in aviculture.

Neophemas—Genus Neophema. Sexual dimorphism occurs in this genus and varies from slight difference in coloration in the Bourke's Parakeet to obvious dimorphism in the Scarlet-chested Parakeet. The Scarlet-chested male has a scarlet chest, whereas the chest of the female is green. In most species the female is duller in color

Barabands, Rock Pebblers, etc.—Genus Polytelis. Members of this genus show some degree of sexual dimorphi The male in this genus is often smaller than the female and the female and young are usually duller in color. female Barabands Parakeet (P. swainsonii) lacks the yellow feathers of the male. In the Rock Pebbler the surface of the male's tail feathers are black, whereas they are margined and tipped in pink in the female. The female Princess of Wales (P. alexandrae) is duller in color and the bill is paler red than that of the male

Kakarikis—Genus Cyanoramphus. Monomorphic.

Crimson Wings—Genus Aprosmictus. The male Crimsom-winged Parakeet is easily distinguished from the female his black mantle.

King Parrots—Genus Alisterus. Sexual dimorphism is present in plumage and beak coloration in some but not all species of King Parrots. Some, but not all, subspecies of Green-winged King Parrots are dimorphic.

Eclectus Parrots—Genus Eclectus. These exhibit striking sexual dimorphism in which the male is a brilliant and the female is red and maroon. This color difference is evident at the time of eruption of the first tail and contour feathers in the baby bird. Both sexes have black down.

Greatbills, Blue Napes, and Muller's Parrots—Genus Tanygnathus. Only the Muller's Parrot (T. mulleri) is dimorphic, with the beak being red in the male and white in the female. The male Greatbill (T. megalory and has a much larger beak than the female.

Fig Parrots—Genus Psittaculirostris. Most are dimorphic in plumage coloration.

Pesquet's Parrot—Genus Psittrichas. Male has a red line behind the eye which is absent in the female.

Table 51-1. SEXUAL DIMORPHISM IN PSITTACINES*9,111,13 Continued

PARROTS OF AFROASIAN DISTRIBUTION **PSITTACIDAE**

PSITTACINAE

Ring-necks—Genus Psittacula. All male birds in this genus have a ring encircling the neck or a wide black mustache ring. In some species this is lacking in the female and immature. Common members of the genus include the Ring-neck Parakeet and the Alexandrine Parakeet. Adult male plumage may not be evident until two and a half years in some individuals. In some species the bill color is different in males and females. For example, the female Mustache Parakeet (P. alexandri fasciata) and Derbyan Parakeet (P. derbyana) have black upper beaks, whereas those of the males are red.

Hanging Parrots—Genus Loriculus. Adult birds are sexually dimorphic in plumage and, in some species, in eye

Vasa Parrots—Genus Coracopsis. Hypertrophy of tissues of the vent is evident in the male and is especially pronounced during the breeding season.

Lovebirds—Genus Agapornis. The commonly available species are monomorphic. Some of the uncommon species of lovebirds, however, show striking dimorphism. For example, the male Madagascar Lovebird (A. cana) has a grey head, whereas that of the female is green. The male Abyssinian Lovebird (A. taranta) has a red patch on the forehead and lores which is absent in the female.

African Grey Parrots—Genus Psittacus. Slight sexual dimorphism is evident in African Greys in coloration. The breast and back of the female is a pale chalky grey, whereas the male is a darker grey. This difference is helpful only when comparing birds from the same region, as color varies geographically.

Senegals and Related Species—Genus Poicephalus. Some members of this genus show marked sexual dimorphism, whereas others are monomorphic. The male Red-bellied Parrot (P. ruficentris) has a deep red-orange breast and abdomen, whereas the female's breast is greyish-brown. The female Ruppells Parrot (P. rueppellii) is more brightly colored than the male, having a bright blue rump patch that is absent in the male.

PARROTS OF SOUTH AMERICAN DISTRIBUTION PSITTACIDAE

PSITTACINAE

Macaws—Genus Ara— Blue & Gold, Scarlet, etc. Monomorphic.

Genus Andorhynchus-Hyacinth. Monomorphic.

Conures—Genus Aratinga—Sun Conure, etc. Monomorphic Genus Pyrrhura—Painted Conure, etc. Monomorphic.

Genus Nandayus-Nanday. Monomorphic.

Genus Enicognathus-Austral and Slender-billed. Monomorphic.

Genus Cyanoliseus-Patagonian. Monomorphic.

Hawkheads-Genus Deroptyus. Monomorphic.

Thick-billed Parrots—Genus Rhynchopsitta. Monomorphic.

Quaker Parakeet—Genus Myiopsitta. Monomorphic.

Mountain Parakeets—Genus Bolborhynchus. Only one species, the Golden-fronted Mountain Parakeet (B. aurifrons), is dimorphic. The male has yellow markings on the lores, forehead, throat, and part of the cheek, whereas the female is predominantly green.

Bee Bee Parakeets—Genus Brotogeris. Monomorphic.

Parrotlets—Genus Forpus. All are dimorphic. In most species the male will have blue markings on the rump and/or wings whereas the female is predominantly green.

Amazon Parrots—Genus Amazona. All species are monomorphic with two exceptions: in the Spectacled Amazon (A. albifrons), the male has red markings on the small upper wing coverts and the edge of the carpus; the female is usually green in this area but may have a limited amount of red. The female Yellow-lored Amazon (A. xantholora) lacks the white on the head and the red markings of the male and is in general more drab-appearing than the

Pionus Parrots-Genus Pionus. Monomorphic.

Pileated Parrot—Genus Pionopsitta. The male has a red head and the female has a green head. This dimorphic coloration is evident in immature plumage. Other members of the genus are monomorphic. Caiques—Genus Pionites. Monomorphic.

Taxonomic system from Forshaw, J. M.: Parrots of the World. New York, Doubleday Press, 1973

Color differences have also been observed from one geographical region to another; therefore, this test is helpful only if the birds are from the same region. In the Double Yellow-headed Amazon (Amazona ochrocephala oratrix) the mature male will usually have a faint red tipping on the feathers of the nape. In Blue-and-Gold Macaws (Ara arauana) the female tends to have heavier feathering on feather lines that cross the bare facial skin patches.

Many birds molt into a prenuptial plumage prior to the breeding season. Slight differences in plumage coloration may be easier to detect at this time of year. 11

In most species that exhibit color dimorphism the immature bird resembles the female. Some species do not exhibit adult dimorphic plumage until 1.5 to 2.5 years of age. Sexual maturity may precede the development of adult male plumage in Ring-neck Parakeets (genus Psittacula). Aviculturists often state that the male must earn his ring. Surgical sexing is the logical alternative to waiting an excessive period of time to distinguish the sexes by plumage. ¹³

VENT SEXING

Palpation or observation of the vent is a rapid and accurate method for sexing some avian species and is widely used for sexing newly hatched poultry and waterfowl. A rudimentary male copulatory organ is present in domesticated fowl, other gallinaceous birds, waterfowl, and ratites. In sexually mature female psittacines the cervix may be observed on the left cloacal wall. 1, 11, 14

In canaries the sex may be determined by examination of the vent during breeding season. The female's vent is more rounded and the abdomen is slightly distended, whereas the male's vent is conical in shape and protruding.

SURGICAL SEXING

Caponization techniques were originally performed by early poultrymen to increase the rate of weight gain and decrease male aggressiveness. Ornithologists utilized laparotomy techniques in the 1950's and 1960's for monitoring seasonal differences in gonadal size and for sex determination. In the past 10 years these techniques have been refined by endoscopy. With the development of rigid endoscopes for arthroscopy, the method has become safer and applicable to smaller species. 6, 10, 17-19

Surgical sexing by laparoscopy has become widely accepted among aviculturists and is the most common method of sex determination available today. It is rapid, accurate, and not dependent on sexual maturity. The risk factor is low if the veterinarian is experienced.

Advantages of laparoscopy include the opportunity to examine other organs and systems as well as direct observation of the gonads (see Chapter 15, Endoscopy). Size and development of the gonads may help to determine readiness for a breeding program. This is especially true of the female, as the presence of large follicles, and in some cases corpus albicans, may indicate that the female is actively cycling. In the male, testicular size is not so obviously an indication of age or sexual maturity. Many birds examined immediately before or after fledging will have testicles comparable in size to those of an adult bird

Technique

Physical examination should precede restraint and induction of anesthesia for endoscopy. The use of anesthetics for laparoscopy is controversial. Surgical sexing can be accomplished rapidly without anesthesia and the bird can be returned immediately to its cage. Small species may be easily restrained. In larger species excessive movement may make the procedure difficult. Stress due to fear is reduced when birds are anesthetized; however, the slight risk of anesthetic death is present. The question of whether or not the bird experiences excessive pain or fear has not been resolved but should be considered by the clinician.

Fasting is not necessary unless excessive amounts of food or water are palpated in the crop. In obese birds fasting is helpful in reducing the size of the proventriculus, which otherwise makes visualization of the gonads difficult (see Chapter 15, Endoscopy).

The bird is positioned in right lateral recumbency, since most species have only one ovary on the left side. An assistant is needed in most cases to hold the wings together over the back. The wings should always be held by the humerus so that any sudden flapping will not result in a fractured elbow. The head and left, or both, legs may be restrained by cords, rubber bands, tape, or Velcro fastening strips on a restraint board. The left leg must be extended caudally and securely fastened to prevent injury. This procedure is accomplished so quickly that supplemental heat is rarely needed.

The site of entry is behind the last rib in a palpable depression located approximately at the midshaft and cranial to the femur. The feathers in this area are plucked and the site prepared with a suitable surgical preparation. Draping is ideal but not necessary.

A skin incision is not necessary when using a sharp trocar, and bleeding is minimized. The trocar, with cannula in place, is punched through the skin and muscular layer, taking care to direct the trocar craniodorsally toward the lung. This allows rapid entrance and a wide margin of safety. The distance between the entry site into the thoracic air sac and any organ is great. If the lung is penetrated, bleeding is minimal and adverse effects are rarely noticed. Direction of the trocar cranially also decreases the chance of penetration of the internal iliac vein. The trocar is removed and the endoscope inserted into the cannula. The air sac wall between the thoracic and cranial abdominal air sacs is visualized and followed caudally until the endoscope is pointed toward the middle or the caudal divisions of the kidney. An area in the air sac that is clear and devoid of vessels is located and the scope is pushed through the air sac. This again allows maximum distance between the site of penetration of the air sacs and the organs beneath. The kidney is followed cranially to the triad of the adrenal gland, gonad, and kidney.

Testicular tissue appears elliptical or cylindrical with a smooth vascular surface (Fig. 51-1). The developing follicles of ovarian tissue in the adult female resemble a cluster of grapes (Fig. 51-2). Both testes and ovaries may be pigmented, especially in white cockatoos. The immature ovary appears as a white blanket with an undulating to smooth surface that spreads over the surface of the kidney and adrenal gland. In order to definitively distinguish an immature ovary from a testicle, the cranial and caudal poles should be observed. A testicle will have rounded poles, whereas the poles of the ovary will be flattened. Sex determination is safe and accurate at a very young age even prior to fledging. The testicle is in many cases larger at the age of fledging than at four to eight months of age. In some birds a small fat pad may cover the gonads, obscuring visualization.

Suture of the entry site is not necessary if the puncture method is utilized. When the leg returns to its normal position, the sliding layers of muscle and skin effectively seal the wound.

Bleeding may be encountered by rupture of a vessel or organ. In most cases the bleeding ceases quickly. If bleeding is excessive the bird should be held in an upright position. This allows collection of blood in the air sacs and prevents asphyxiation. Subcutaneous emphysema occurs rarely and in most cases is self-limiting. Post-surgical infections are very rare, even when only cold sterilization procedures are used. Obstruction of vision is the most common reason for failure to determine sex (see Chapter 15, Endoscopy). This method can be



Figure 51-1. Through the endoscope, the mature testicle appears elongated and smooth with evidence of vascularization. (Courtesy of W. Satterfield.)

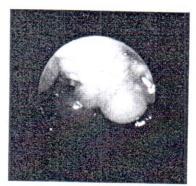


Figure 51–2. The developing follicles on a mature ovary give an appearance of a cluster of grapes. (Courtesy of W Satterfield.)

modified for surgical sexing utilizing the oto-scope. $^{8, 12}$

Safety and accuracy of surgical sexing vary significantly with the experience of the endoscopist. Mortality rates for surgical sexing by an experienced endoscopist are very low, ranging from 0.2 to 1.0 per cent. As with diagnostic laparoscopy, the veterinarian should first practice with dead birds to study the anatomy and learn to handle the equipment. Practice with birds such as pigeons should precede attempted sexing of clients' birds.¹

Surgical sexing is widely applied to psittacine collections with a high degree of success and few complications. Fertile eggs have been laid on many occasions within a month following surgical sexing.

GENETIC SEXING METHODS

Avian chromosome analysis techniques have evolved slowly over the last 15 years. While highly accurate and applicable to any bird regardless of age or condition, these methods are expensive and of limited availability.*^{1, 5, 15, 20, 21}

The diploid (2n) chromosome number in birds ranges from 52 to 92. The female bird is homogametic (ZZ), whereas the male is heterogametic and is designated (ZW). Avian W chromosomes are smaller than the Z chromosome. Karyotypic evaluation is accomplished by matching chromosomes based on gross morphologic characteristics such as length, shape, centromeric position, and specific staining qualities.

Karyograms are prepared by three basic methods: (1) squash preparations of feather pulp cells from pin feathers, (2) culture of peripheral

^{*}Marc Valentine, Avian Genetic Sexing Laboratory, Memphis, TN. Call ahead for test kit. (901) 323-4045.

blood leukocytes, or (3) culture of feather pulp cells. Mitotic cells are arrested in late prophase or metaphase, stained, and photographed, allowing pairing of chromosomes and identification of sex chromosomes.

The squash method is simple; however, the cells are often traumatized, resulting in loss of chromosomes. Staining is also variable, and artifacts from debris may interfere with the test. For leukocyte culture, approximately 2 ml of heparinized blood is required. The blood is cultured for 72 hours and the cells are arrested with colcemid. Staining of these preparations is more consistent, and loss of chromosomes is less frequent. The difficulty of culturing avian blood is the primary disadvantage in this procedure. Tissue culture of feather pulp markedly improves the morphology of the metaphase cells and the staining properties of the preparations. This method allows very accurate sex determination at any age and is currently available commercially.* The need for close proximity to the laboratory or rapid shipping time coupled with the expense and time involved makes this method impractical for routine sex determination.

Specific banding and staining techniques are under active investigation and may prove to be a viable technique for sex determination in the future. Chromosome maps of unique fluorescent surface patterns can be produced, evaluated, and documented by special staining and fluorescent techniques. The W chromosome of birds is composed almost entirely of constitutive heterochromatin similar to the centromeres of all chromosomes. The uniform staining of the W chromosome enhances its identity from other macrochromosomes with large nonfluorescing areas.

HORMONAL SEXING METHODS

Fecal Steroid Analysis

Sex determination through measurement of sex steroid hormones provides a viable alternative to invasive techniques. These techniques have been adapted from human pregnancy testing methods. ^{1, 2, 16}

Total excretory estrogen (E) and testosterone (T) can readily be measured from the mixed fecal droppings of unrestrained birds. Only the urate portion is needed, but both feces and

The obvious advantage of this method is in the fact that the birds require no restraint and readily provide the necessary test material. The disadvantages include the inability to sex immature or inactive birds. Seasonal inactivity as well as poor health affect the accuracy of this method. Accuracy is 95 to 99 per cent in mature active birds; however, 20 to 40 per cent of birds tested at the San Diego Zoo could not be sexed. The Avian Hormone Laboratory at Michigan State University discontinued offering the test because 33 per cent of the samples were unsuitable for accurate sex determination. 1, 16

Egg Waste Estrogen Analysis

A method developed at the San Diego Zoo in 1983 allows sexing of hatchlings by steroid analysis of fecal material present in the egg at hatching. Active steroidogenesis and sex hormone production occur in embryonic gonads, and these hormones are present in the urates excreted immediately prior to or during hatching. Mixed urates and feces are collected after hatching, allowing noninvasive sex determination of hatchlings. Estradiol fractions are isolated and quantified by high performance liquid chromatography. This procedure is costly and is not currently available commercially.^{1,3}

Plasma Hormone Analysis

Plasma hormone ratios can also be used for sexing birds. Testosterone, free estriol, and estriol levels are determined by RIA analysis of plasma. The ratio of these hormones is used to determine sex in the same way as fecal steroids. Blood may be collected in two to six heparinized hematocrit tubes and spun down, and the tubes are snapped and capped with clay. Serum may be submitted in serum separator tubes. The

urates are collected for a two- to four-hour period each morning for two or three days. Samples are frozen and shipped to the lab for analysis. Estrogen and testosterone levels are determined by radioimmunoassay (RIA) methods that are sensitive to picogram (10 to 12) changes in concentration. The relative amounts of estrogen and testosterone are expressed in a simple E/T ratio. At the San Diego Zoo mature female parrots are identified by a relatively high ratio (2.78 \pm 0.58), and mature males are denoted by a low ratio (0.64 \pm 0.13). The values differ for each laboratory. Fecal steroid analysis is not currently commercially available.

^{*}Marc Valentine, Avian Genetic Sexing Laboratory, Memphis, TN. Call ahead for test kit. (901) 323—4045.

samples are frozen and shipped with cool packs to the laboratory. The samples are stable for at least six days in transit. Results are reported in approximately one to two weeks.*7

Advantages of plasma hormone analysis over fecal steroid analysis include reduced chance of contamination and less dependence on light cycles and seasonal activity of the bird. While hormone levels are lower in immature birds. the ratio of hormones may still be an accurate indication of sex; however, there is some controversy over the accuracy of this method. Stress and surgical and anesthetic risks are obviously reduced.

Genetic and endocrine sexing methods are specialized, expensive, and still only experimentally applied. Extensive training and special equipment are required, and some methods are very time consuming. Genetic determinations have the distinct advantage of being highly accurate in sexing birds of any age or condition. Endocrine evaluations offer the most direct assessment of functional activity of reproductive organs. Surgical sexing remains the most practical and readily available method of sex determination in monomorphic avian species.

REFERENCES

- 1. Bercovitz, A. B.: Annotated Review of Avian Sex Identification Techniques. In Burr, E. (ed.): Companion Bird Medicine. Ames, IA, Iowa State University Press, in press.
- 2. Bercovitz, A. B.: Fecal steroid analysis: A non-invasive approach to bird sexing. Proceedings of the American Federation of Aviculture, Veterinary Seminar, San Diego, CA, 1981.
- 3. Bercovitz, A. B.: Endocrine fecology of immature birds. Watchbird Mag., Volume 11, No. 2, 1984.
- 4. Biederman, B. M., and Lin, C. C.: A leukocyte culture

- and chromosome preparation technique for avian species. In Vitro, 18:415-418, 1982.
- 5. Bloom, S. E.: Current knowledge about the avian W chromosome. Bio. Sci., 24:340-344, 1974.
- 6. Bush, M., et al.: Sexing birds by laparoscopy. Int. Zoo Yearbook, 18:11, 1978.
- 7. Davis, S.: Personal communication, 1984.
- Fletcher, K.: Surgical Sexing of Birds of Prey. AAZPA Regional Conference Proceedings, 1981, pp. 432-
- 9. Forshaw, J. M.: Parrots of the World. New York, Doubleday Press, 1973.
- 10. Harrison, G. J.: Endoscopic examination of avian gonadal tissue. VM/SAC, 73:479–484, 1978.

 11. Harrison, G. J.: Personal communication, 1984.
- 12. Ingram, K. A.: Otoscopic technique for sexing birds. In Kirk, R. (ed.): Current Veterinary Therapy VII. Philadelphia, W. B. Saunders Company, 1980.
- 13. Low, R.: Parrots, Their Care and Breeding. Poole, Dorset, England, Blandford Press, 1980.
- 14. Masui, K.: The rudimentary copulatory organ of the male domesticated fowl with reference to the sexual differentiation of chickens. In Masui, K. (ed.): Sex Determination and Sexual Differentiation in the Fowl. Ames, IA, Iowa State University Press, 1967, pp. 3-15.
- 15. Mengden, G. A., and Stock, A. D.: A preliminary report on the application of current cytological techniques to sexing birds. Int. Zoo Yearbook, 16:138-141, 1976.
- 16. Nachreiner, R.: Personal communication, 1984.
- 17. Satterfield, W. C.: Diagnostic laparoscopy in birds. In Kirk, R. (ed.): Current Veterinary Therapy VII. Philadelphia, W. B. Saunders Company, 1980.
- 18. Satterfield, W. C., and Altman, R. B.: Ayian sex determination by endoscopy. Proceedings of the American Association of Zoo Veterinarians, 1977, pp. 45 - 48.
- 19. Thomas, B.: Otoscopic sexing of birds: Emphasis on identification of the gonads. Auburn Vet., 39(1):23-26, 1983.
- 20. Toone, W. D.: Improvements in cytogenetic techniques for gross karyotypic and morphological studies of avian chromosomes. Proceedings of the American Federation of Aviculture Veterinary Seminar, San Diego, CA, 1981.
- 21. Van Tuinen, P., and Valentine, M.: A non-invasive technique of avian tissue culture (feather pulp) for banded chromosome preparations. Mammalian Chromo. Newsletter, 23(4):182-186, 1982.

^{*}National Development & Research, 4850 156 Ave. N.E., #45, Redmond, Washington 98052