

SEXING MATURE COLUMBIFORMES BY
CLOACAL CHARACTERS

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WHILE there is a pronounced sexual dimorphism or dichromatism in some species of the order Columbiformes, in most others there is not. Until recently we have known of no simple and reliable method for sexing living individuals of these latter species. Various techniques which have been used are: observation of slight quantitative differences in the coloration on the head, neck, and breast of the Mourning Dove, *Zenaidura macroura* (Petrides, 1951); slight differences in the contour of the head and neck of the domestic pigeon, *Columba livia*, (Levi, 1941); differences in behavior; and others. For a number of years, sexing by internal examination of gonads (by laparotomy) has been frequently used in the department of Genetics, University of Wisconsin. This is a delicate and time-consuming operation. Iwata (1926) described a method in which differences of the shape of the lips of the vent of *Columba livia* are used to distinguish the sexes. This technique has not to our knowledge been verifiable in this country (Levi, 1941).

The objectives of this paper are three-fold: (1) to describe a simple method for sexing living adult doves and pigeons by cloacal characters and to present data on the reliability of the method; (2) to list the diverse species in which we have tried this method; and (3) to present data from brief experiments which indicate that the development of these characters is under endocrine influence. We believe the method to be quite reliable and as simple to use as the cloacal examination method for sexing ducks (Hochbaum, 1948) or the widely used method of determining the age of birds by the bursa of Fabricius (Gower, 1939).

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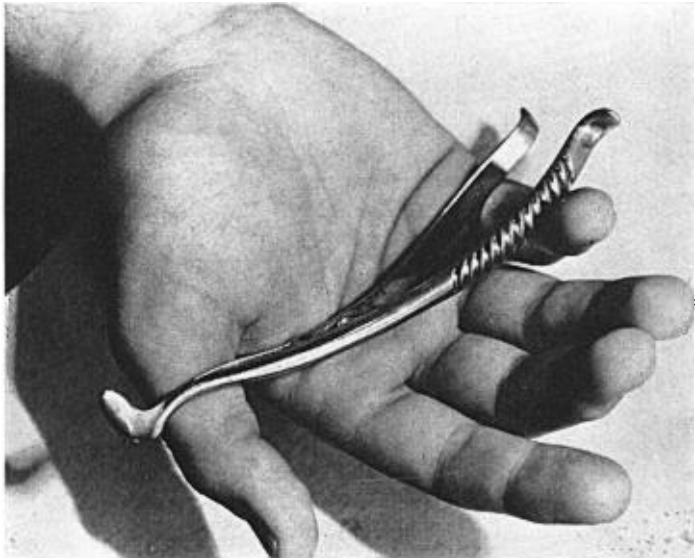
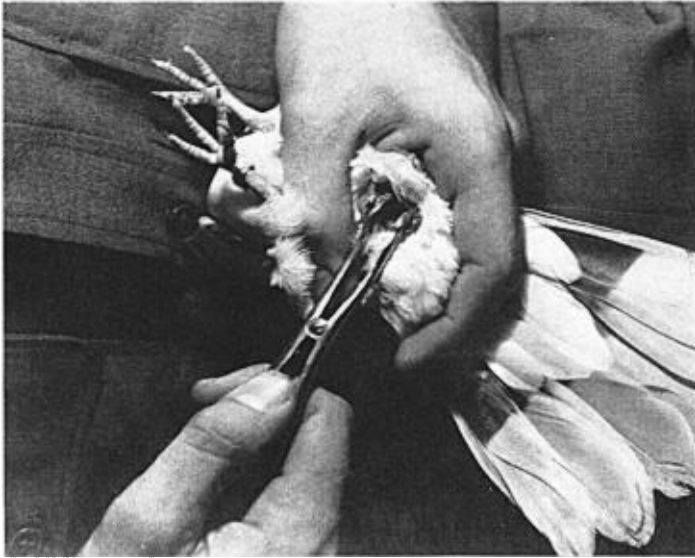
Procedure.—The majority of birds examined belonged to the Department of Genetics of the University of Wisconsin. Additional birds were examined in the two zoos previously mentioned. Most of the Mourning Doves were trapped or collected in the field in southern Wisconsin.

Briefly, the method involves insertion of a modified nasal speculum into the vent to allow identification of cloacal features. The sexing method was suggested by Elwood Briles and first used by Jeff Faust, Foreman in the Department of Genetics, University of Wisconsin. If this method has been used previously, we are unaware of it.

Males possess two conical papillae, one on each side of the cloaca, which terminate the sperm ducts (*vasa deferentia*). Depending on the size of the birds, these papillae vary in length from one to two or three millimeters in the 31 species and hybrids we have examined. They are lacking in females, and the oviduct opening, often of a whitish color, may be seen in the wall of the cloaca on the left side.

The sexing instrument is shown in plate 13. This may be a nasal speculum purchased at a medical supply store. The jaws are ground down on an emery wheel and are three to five millimeters in width and from 17 to 25 millimeters in length. Care should be taken to grind or file the jaws smooth to prevent damage to the cloacal tissue.

For examination, a bird is held under a light, head downward and feet toward the operator. Plate 13 demonstrates this pictorially, although in order to show the insertion of the instrument, the bird is held more horizontally in the picture than it would be in practice. A few small feathers around the vent are plucked. The observer's left forefinger holds back the rectrices and under-tail coverts while the thumb restrains the feathers ventral and anterior to the vent. The head of the instrument is inserted gently to a depth of about one centimeter and directed to the left (to ten o'clock considering the cloaca as a horizontal clock face). The jaws are expanded and at the same time a slight forward and upward pressure (dorsal and posterior to the bird) is applied. This has the simultaneous effects of spreading the lips of the vent, pushing forward (dorsad) the left and slightly posterior wall of the cloaca, and erecting the papilla if the bird is a male. To view the opposite side (not necessary for the sexing process), the instrument is turned in the opposite direction and handled in the same manner. The cloacal structures are shown in plate 14.



The Sexing of Pigeons and Doves. (*Top*) Method of holding bird for examination. (*Bottom*) Modified nasal speculum used for spreading the vent.



The Sexing of Pigeons and Doves. (*Top*) Close-up, showing the cloacal papillae of the male. (*Bottom*) Close-up, showing the oviduct opening of the female.

If the breeding status of the bird is not known, extreme care should be exercised in inserting the instrument, since a female with an egg in the oviduct might be injured. Eggs can usually be palpated, and females during the breeding season should be so examined if there is any doubt.

We were unable to demonstrate the presence of the cloacal papillae or oviduct opening in immature birds. The possibility occurred to us that the development of these characters could be under the influence of the reproductive hormones. To test this possibility, several experiments with recently fledged domestic pigeon squabs (about five weeks of age) were conducted. The birds were of a strain which carried a mutant color gene described by Hollander (1942) and termed "faded" (ST^F). The gene is sex-linked and dominant to the blue bar of "wild type." It forms the basis of a permanent autosexing strain of birds which can be sexed by plumage. The males are predominantly white with dark flecks whereas the females are nearly normal in color. The following experiments involving hormone treatments were conducted with these squabs of known sex.

Experiment 1.—One female was injected in the pectoral muscles daily with 0.25 cc. of testosterone propionate solution (5 mg. of powdered hormone in 1 cc. of Sesame oil). One male was injected daily with 0.5 cc. of estradiol benzoate solution (1.66 mg. of hormone per cc. of Sesame oil). The birds were examined daily for development of the cloacal characters during the 18 days of injection.

Experiment 2.—Two females were implanted with diethyl stilbesterol pellets under the skin of the neck. One male was given daily, intramuscular injections of 0.25 cc. of testosterone propionate of the concentration described in Experiment 1. Injections were continued 13 days. All birds were examined daily during the period for development of cloacal characters.

Experiment 3.—Three males were implanted with diethyl stilbesterol pellets, two birds with two pellets, and one bird with one pellet. The following day, one of the birds carrying two pellets died. The other two were examined daily for 12 days.

Experiment 4.—Two females were injected daily with 0.5 cc. of testosterone propionate with the concentration described above. This is a doubling of the doses previously described. The birds were injected for eight days and examined daily.

Reliability of the technique.—The sex of 132 birds of ten species and a backcross hybrid from a species cross was known either from

previous behavior, sex-linked matings, or breeding activity. Seventeen Mourning Doves (*Zenaidura macroura*) were collected during the course of other work and sexed by this method before internal examination. The sex of 91 per cent (120 birds) of these birds of known sex was correctly indicated by our cloacal method; our detection of the sex of 12 birds (9 per cent) was uncertain, and none was incorrectly sexed. Another group of 203 individuals from 30 different species and hybrids whose sex was not previously known was also examined. It was possible to assign the sex of 188 or 93 per cent of these, while the sex of 15 was uncertain. These details are summarized in table 1.

We believe that some of the birds we examined were immature which may have lowered the success in determining their sex. Since the "pilot" study, several hundred individuals of *C. livia*, *Streptopelia risoria*, and their hybrids and backcross hybrids with other species have been sexed by this cloacal method. Accuracy approached 100 per cent when the birds were healthy and mature.

A number of birds belonging to seasonally breeding species (e.g. *Columba fasciata*, *Z. macroura*, and others) were examined in winter and the cloacal characters described in this paper were recognizable. However, this is not a reliable indication as to whether or not there is regression of the characters in the sexually quiescent season. These birds are housed in a building where artificial lights are frequently kept burning longer than the normal photoperiod for the season. As a result, there is some stimulation of breeding physiology since some individuals show tendencies to breed throughout the year. This aspect should be studied further.

If other areas of the cloaca are examined, occasionally a second set of minute pimple-like processes may be seen in addition to the larger papillae in the males and the left oviduct opening in the females. This is more typical of the larger species, especially *C. livia*. These are apparently the urinary papillae terminating the ureters. They should cause no confusion since they are much smaller than the prominent terminal papillae of the *vasa deferentia* and should not cause one to mistake a female for a male.

Hormone influence.—The results of the experiments with hormones are shown in table 2. It is apparent from the results of Experiment 2 that precocious development of the papillae in the male and oviduct opening in the female were stimulated by androgen and estrogen treatment, respectively. It may also be seen from the results of Experiments 1 and 4 that growth of papillae in the females was

TABLE I
ACCURACY OF SEXING AND LIST OF SPECIES EXAMINED BY CLOACAL METHOD

| Species | Common name | Sex known | | Sex unknown | | Uncer- tain |
|-----------------------------------|------------------------------|--------------------|----------------|------------------|--------------------|----------------|
| | | Correctly Sexed | Uncer- tain | Sexed as male | Sexed as female | |
| <i>Columba fasciata</i> | Band-tailed Pigeon | — | — | 0 | 3 | 0 |
| <i>Columba guinea</i> | Triangular-spotted Pigeon | — | — | 5 | 5 | 2 |
| <i>Columba livia</i> | Domestic Pigeon or Rock Dove | 35 | 6 | 3 | 11 | 1 |
| <i>Columba palumbus</i> | European Wood Pigeon | — | — | 1 | 1 | 0 |
| <i>Columba rufina</i> | Rufous Pigeon | — | — | 0 | 1 | 0 |
| <i>Columba guinea-livia</i> | Backcross hybrids | 26 | 2 | 9 | 5 | 1 |
| <i>Streptopelia bitorquata</i> | Philippine Turtle Dove | — | — | 2 | 1 | 0 |
| <i>Streptopelia capicola</i> | Cape Turtle Dove | — | — | 8 | 3 | 1 |
| <i>Streptopelia chinensis</i> | Pearl-necked Dove | 5 | 0 | 7 | 0 | 0 |
| <i>Streptopelia orientalis</i> | Chinese Turtle Dove | — | — | 2 | 1 | 0 |
| <i>Streptopelia risoria</i> | Ring-necked Dove | 17 | 0 | 15 | 5 | 0 |
| <i>Streptopelia semitorquata</i> | African Turtle Dove | 3 | 0 | 3 | 2 | 1 |
| <i>Streptopelia senegalensis</i> | Senegal Turtle Dove | 2 | 0 | 29 | 9 | 2 |
| <i>Streptopelia tranquebarica</i> | Dwarf Turtle Dove | 7 | 2 | — | — | — |
| <i>Streptopelia turtur</i> | European Turtle Dove | — | — | 0 | 1 | 1 |
| <i>Caloenas nicobarica</i> | Nicobar Dove | — | — | 2 | 2 | 0 |
| <i>Chalcophaps indica</i> | Green-winged Dove | 0 | 1 | — | — | — |
| <i>Gallinolumba lusonica</i> | Bleeding Heart Pigeon | — | — | 0 | 1 | 0 |

TABLE I—Continued
ACCURACY OF SEXING AND LIST OF SPECIES EXAMINED BY CLOACAL METHOD

| Species | Common name | Sex known | | Sex unknown | | |
|------------------------------|----------------------------|--------------------|----------------|------------------|--------------------|----------------|
| | | Correctly Sexed | Uncer- tain | Sexed as male | Sexed as female | Uncer- tain |
| <i>Geopelia humeralis</i> | Bar-shouldered Dove | — | — | 1 | 4 | 0 |
| <i>Leptotila jamaicensis</i> | Violet Dove | — | — | 1 | 0 | 0 |
| <i>Ocyphaps lophotes</i> | Australian Crested Dove | — | — | 2 | 5 | 1 |
| <i>Phaps chalcoptera</i> | Bronze-winged Pigeon | — | — | 2 | 2 | 0 |
| <i>Phaps elegans</i> | Brush Bronze-winged Pigeon | — | — | 1 | 1 | 1 |
| <i>Scardafella inca</i> | Inca Dove | — | — | 1 | 0 | 0 |
| <i>Zenaidura asiatica</i> | White-winged Dove | — | — | 2 | 1 | 0 |
| <i>Zenaidura aurita</i> | Martinique Dove | 3 | 0 | 0 | 1 | 0 |
| <i>Zenaidura auriculata</i> | Bronze-necked Dove | — | — | 2 | 0 | 1 |
| <i>Zenaidura graysoni</i> | Grayson's Dove | 2 | 0 | 2 | 2 | 0 |
| <i>Zenaidura macroura</i> | Mourning Dove | 20 | 1 | 4 | 2 | 2 |
| <i>Ducula spilorrhoa</i> | White Fruit Pigeon | — | — | 1 | 2 | 1 |
| <i>Goura cristata</i> | Göira or Crowned Pigeon | — | — | 0 | 2 | 0 |
| Total individuals | | 120 | 12 | 105 | 83 | 15 |
| Per cent | | 91 | 9 | 93 | | 7 |

TABLE 2
SEX HORMONE STIMULATION OF CLOACAL CHARACTERS IN IMMATURE PIGEONS

| | | Androgen treatment | | | | Estrogen treatment | |
|-----------------|-----|------------------------|------------------|-----------------|-----|------------------------|-------------------------------|
| Experi- ment | Sex | Length of treatment | Results | Experi- ment | Sex | Length of treatment | Results |
| 1 | ♀ | 8 days | 2 large papillae | 1 | ♂ | 21 days | no change |
| 2 | ♂ | 5 days | 2 papillae | 2 | ♀ | 6 days | oviduct open- ing apparent |
| 4 | ♀ | 7 days | 2 papillae | 2 | ♀ | 13 days | oviduct open- ing apparent |
| 4 | ♀ | 7 days | 2 papillae | 3 | ♂ | 43 days | no change |
| | | | | 3 | ♂ | 43 days | no change |

stimulated by androgen injection! However, two efforts (Experiments 1 and 3) to induce growth of oviduct openings in the males by estrogen treatment were unsuccessful.

It is not within the scope of this paper to discuss other aspects of these cloacal characters such as the embryological. However, our findings of the relationship of these characters to their respective sex hormones further confirms the reliability of their use in distinguishing the sex of adult members of the order Columbiformes.

Summary.—Conical papillae terminating the *vasa deferentia* are situated on each side of the cloaca of males of 30 species of Columbiformes. These are lacking in females which have a left oviduct opening. These characters formed the basis of a simple sexing method which allowed sexing accuracy well above 90 per cent for the species studied. None was incorrectly sexed. Premature development of the papillae both in male and female squabs was stimulated by androgen injection; premature development of the oviduct opening was stimulated by estrogen in the females but not in males.

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