A COMPARATIVE KARYOTYPE STUDY IN FOURTEEN SPECIES OF BIRDS

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A considerable amount of work has been available in earlier literature on the chromosomes of birds which were done with classic testis-section methods. Surprising improvement of cytogenetic techniques has lately made possible analyses of the chromosomes more precisely and reliably than those done with old techniques. Recently, many species of birds were reinvestigated with modern techniques involving tissue culture and hypotonic-colchicine pretreatment procedures with new findings, particularly on the chromosome number and sex-determining mechanism.

Generally, avian karyotypes are characterized by the presence of macrochromosomes and microchromosomes, distinctly different in size and shape. Interest has further been focussed on genetical female heterogamety by the occurrence of a ZZ-ZW sex mechanism. Since technical difficulties are always a bar to the exact demonstration of the microchromosomes even with current techniques, accounts on avian karyotypes together with the sex-determination mechanism have now remained rather incomplete for many forms of birds. The present study was undertaken, based on feather-pulp cultures, non-culture marrow cells and direct squashes, to deal with the morphology of chromosomes and sex-mechanism in 14 species of birds.

MATERIALS AND METHODS

Birds subjected to the present chromosome study are the ostrich (*Struthio camelus camelus*), the herring-gull (*Larus argentatus*), the Davison's ground-thrush (*Turdus sibiricus davisoni*), the Japanese scops owl (*Otus scops japonicus*), the reeves' pheasant (*Syrnaticus reevesii*), the diamond dove (*Geopelia cuneata*), the white dove (*Streptopelia risoria* var. *alba*), the common pigeon (*Columba livia domestica*), the ring dove (*Streptopelia risoria*), the eastern greylage goose (*Anser anser*), the Pacific white-front goose (*Anser albifrons*), the Indian goose (*Eulabeia indica*), the eastern grey heron (*Ardea cinerea*), and the Malay gallinule (*Porphyrio poliocephalus viridis*). They are listed in Table 1.

The chromosome studies were carried out with feather-pulp cultures in the ostrich, the herring-gull, the eastern greylage goose, the Pacific white-front goose, the Indian goose, the eastern grey heron and the Malay gallinule according to the method of

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Sasaki *et al.* (1968). Growing tails or wing-feather of living birds were obtained from several Zoos. The feather-pulp specimens were washed in Ringer's solution, cut into shreds with sharp scissors and planted directly on the glass surface of the culture bottle with plasma clots. Cultivation was made in medium TC-109 supplemented with 15% inactivated bovine serum at 37°C. After 6 to 8 days, cultures were exposed to colcemid (0.1 µg/ml) for final 6 hours of incubation. After being dislodged with trypsin solution, the cells were treated with hypotonic KCl solution (0.075M) for 15 minutes at 37°C and fixed with methanol-acetic acid (3 : 1).

In the Davison's ground-thrush, the Japanese scope owl and the domestic pigeon, bone marrow cells were subjected to chromosome study: the cells obtained from the thigh bone were immediately placed in culture medium with 15% bovine serum, and treated with colchicine at 37°C for 2 hours. Following the hypotonic treatment with KCl solution for 15 minutes at 37°C, the cells were fixed with metanol-acetic acid (3 : 1).

Chromosome slides were made by the air-drying method with Giemsa staining in both cultured and non-cultured specimens. Direct squashes prepared with spleen and marrow tissues served for study in the diamond dove, the ring dove and the white dove.

DESCRIPTION OF DATA WITH REMARKS

The results of the present investigations are given in each species along with some remarks. The variation in number of the chromosome in the same species may be attributable, for the most part, to the difficulty in exact counting of some small-sized chromosomes. Metaphase chromosomes from each species were photographed and karyotyped. At least three well-spread metaphases were used for chromosome analyses in each species, with special attention to large-sized chromosomes. Figures 1 to 11 showed the chromosomes of 11 species aligned in a serial descending order of size, particularly in larger chromosomes. In each species, the chromosome complements are represented by 8 to 9 pairs of macrochromosomes and 24 to 38 pairs of microchromosomes. A difficulty occurred always for the exact determination of the number and morphological identification of the microchromosomes.

The herring-gull (Larus argentatus)

A pioneer and salient chromosome study of the gull (*Larus argentatus vegae* Palmen) was done by Oguma (1937) reporting 66 chromosomes which consisted of 18 macrochromosomes and 48 microchromosomes. The present study was made with feather-pulp cultures of a herring-gull of unknown sex. Chromosome counts varied from 56 to 72. Figure 1 shows a karyotype with 66 chromosomes, 20 macrochromosomes and 46 microchromosomes. The macrochromosomes were arranged into 9 discrete pairs except 2 submetacentrics. Nos. 1 and 2 pairs are represented by large submetacentrics. The centromere of no. 2 is more median than that of no. 1. No. 3 pair is of acrocentric nature having small short arms. No. 4 chromosomes are subtelocentrics. Nos. 5 and 6 chromosomes are telocentric in appearance, corresponding in length to the long arm of no. 4. No. 7

chromosomes are subtelocentrics. Nos. 8 and 9 pairs are submetacentric chromosomes, in which short arms of no. 9 are longer than those of no. 8. The occurrence of at least 36 microchromosomes were determined in well-spread metaphases.

The presence of 2 unpaired submetacentric chromosomes is of particular interest in relation to sex-mechanism of this sex-unknown specimen. Though no final statement is made at the moment, most probably they correspond to the ZW chromosomes. The larger one ranks in size between nos. 4 and 5 chromosomes, while the smaller one corresponds nearly to no. 9 in size and shape.

The Davison's ground-thrush (Turdus sibiricus davisoni)

Bone marrow cells from a male bird provided 18 well-defined metaphases for study. The chromosome number varied from 74 to 82. The chromosome complement showed 14 macrochromosomes and 60 to 68 microchromosomes. A karyotype with 80 chromosomes is presented in Figure 2. No. 1 submetacentric pair is easily distinguishable from the others by size. Nos. 2 and 3 chromosomes are sub- and telocentric in appearance, respectively. Chromosomes no. 4 are submetacentric and no. 5 are of telocentric nature. Chromosomes forming nos. 6 and 7 pairs are similar in length, but well distinguishable from each other, the former being submetacentric while the latter telocentric. At least 66 telocentric microchromosomes were observed to occur in good metaphases. The identification of the sex chromosomes has remained for future study.

The reeves' pheasant (Syrmaticus reevesii)

Several species of the Phasianidae (*Phasianus*, *Syrmaticus*, *Chrysolophus* and *Gennaeus*) were chromosomally studied by older testis-section method (Yamashina 1943, 1946), and a karyological similarity with 82 chromosomes was reported in them. Based on tissue cultures, Takagi and Makino (1966) demonstrated a Z-W sex-mechanism in the female of *Phasianus colchicus karpowi* reporting 20 large-sized chromosomes.

The chromosomes were studied in 14 well-spread metaphases from feather-pulp cultures of a male reeves' pheasant. The chromosome number showed a variation ranging from 72 to 82. The complement with 82 chromosomes consisted of 16 macrochromosomes and 66 microchromosomes (Fig. 3). No. 1 pair is represented by submetacentric elements. The chromosomes forming nos. 2, 3, 5, 6, 7 and 8 pairs are of telocentric structure in general appearance. They showed a gradual seriation in size. No. 4 chromosomes are meta- or submetacentric. At least 54 microchromosomes of telocentric nature were detectable in good metaphases.

According to Takagi and Makino (1966), the Z chromosome of *Phasianus* is represented by a metacentric element ranking in size between the 3rd and 4th pairs. It is therefore very likely that no. 4 pair may constitute a Z-Z complex in this species also.

The ostrich (Struthio camelus camelus)

Sasaki *et al.* (1968) studied the chromosomes of the ostrich based on feather-pulp cultures, reporting 72 to 80 chromosomes characterized by 6 large pairs.

The present study was carried out in three ostriches of unknown sex with chromosome slides made from feather-pulp cultures. The chromosomes ranged in number from 74 to 84 in the three individuals. Karyotype analyses of cells with 80 chromo-

somes revealed the occurrence of 16 macrochromosomes (in 8 pairs) and 64 microchromosomes (Fig. 4). Eight macrochromosome pairs were divided into 2 submetacentric and 6 telocentric pairs. Nos. 1 and 2 chromosomes are of submetacentric structure, different in size. Nos. 3 to 8 pairs are represented by telocentrics. No. 3 chromosomes are identifiable in the telocentric elements because of the largest size, while the chromosomes of nos. 4 to 6 are indistinguishable from one another due to the absence of characteristic structural configuration. Nos. 7 and 8 are the smallest telocentrics among the macrochromosomes. These smallest elements were described as members of the microchromosomes by Sasaki *et al.* (1968). At least 58 microchromosomes were well detected in the complement.

The Japanese scops owl (Otus scops japonicus)

Krishan *et al.* (1965) reported 82 to 84 chromosomes in feather-pulp cells and cultured leucocytes of the great horned owl (*Bubo v. virginianus*) in both sexes. According to them the fourth chromosome was the Z chromosome which was nearly identical in size with the 7th pair, and metacentric in nature.

Bone marrow cells of a male *Otus scops japonicus* provided 16 well-defined metaphases available for the present study. The number of chromosomes was found in a range from 70 to 78. Figure 5 shows a karyotype with 76 chromosomes consisting of 18 macrochromosomes and 58 microchromosomes. Nos. 1 to 3 pairs are represented by subtelocentric elements different in size. They had a minute short arm in each. Nos. 4 and 5 are submetacentric in appearance; no. 4 has the centromere locating more medially than no. 5. No. 6 are telocentric. Nos. 7 to 9 pairs are represented by submetacentric elements, no. 9 being well-identified on account of their smaller size. The presence of at least 52 microchromosomes were noted in the complement. In comparison with the chromosomes of the great horned owl (Krishan *et al.* 1965), the sex chromosomes of the present species is represented, most probably, by the 4th pair, though no final statement can be made with the present material.

The domestic pigeon (Columba livia domestica), diamond dove (Geopelia cuneata), ring dove (Streptopelia risoria) and white dove (Streptopelia risoria var. alba)

Yamashina and Makino (1946) and Makino *et al.* (1956) studied the chromosomes of the pigeon and dove by means of the classic testis-section method. They noted 16 macrochromosomes. Galton and Bredbury (1966) recognized 18 macrochromosomes in the pigeon. The following observations were carried out with air-dried marrow cells for the domestic pigeon of both sexes, and squashed marrow and spleen cells for a female diamond dove, a male and a female ring doves, and a female white dove.

The chromosomes of the domestic pigeon ranged in number from 77 to 81. Rough chromosome countings were given in other species, because of some difficulty in examination of microchromosomes. Figures 6 to 8 are karyotypes derived from the female specimens of the domestic pigeon, the diamond dove and the white dove. Comparison of the chromosomes between the ring dove and the white dove revealed a morphological similarity, especially in the macrochromosomes. The occurrence of 18 macrochromosomes was evident in the pigeon and doves. In general, the chromosomes forming nos. 1 to

Figs. 1-5. Karyotype of *Larus argentatus*, with 66 chromosomes. 2. Karyotype of *Tudus sibiricus davisoni*, with 80 chromosomes. 3. Karyotype of *Syrnaticus reevesii*, with 82 chromosomes. 4. Karyotype of *Struthio camelus camelus*, with 80 chromosomes. 5. Karyotype of *Otus scops japonicus*, with 76 chromosomes.

5 pairs are similar in appearance among the pigeon and doves. The larger 3 pairs can easily be recognized on the basis of their size and shape. The chromosomes of nos. 1 and 2 pairs are of submetacentric nature, while no. 3 pair is represented by subtelo- or telocentric elements. Nos. 4 and 5 are submetacentric in appearance, no. 5 of the domestic pigeon having the centromere more medially located than no. 4.

Interesting are nos. 6 and 7 chromosomes, since they are differential among the three species. No. 6 chromosomes are telocentric in the domestic pigeon, submetacentric in the diamond dove, and metacentric in the white dove.

On the basis of karyotype analysis, the Z chromosome identified for the domestic pigeon was a metacentric element ranking in size between the 3rd and the 4th chromosomes, while that of the white dove corresponded to the 3rd chromosome. The Z chromosome identified for the diamond dove is represented by a submetacentric chromosome nearly identical in size with no. 3 chromosomes. The W chromosome was shown

by the smallest element in the macrochromosomes, being metacentric in the domestic pigeon, while telocentric in the diamond dove and the white dove.

The Z-chromosome identified for the domestic pigeon and dove in the present study is nearly concordant with that reported by Yamashina and Mahino (1946) and Makino *et al.* (1956) through the classic method.

The eastern greylage goose (Anser anser), the Pacific white-front goose (Anser albifrons) and the Indian goose (Eulabeia indica)

The chromosomes were studied in 38 well-spread metaphases from feather-pulp cultures of the above mentioned three sex unknown species of the Anatidae. The chromosomes of the Anatidae were studied by Yamashina (1951, 1952) by means of the old testis-section method.

The chromosomes of three species of the Anatidae here concerned were nearly the

Figs. 6-11. 6. Karyotype of *Columba livia domestica*, with 80 chromosomes. 7. Karyotype of *Geopelia cuneata*, with 72 chromosomes. 8. Karyotype of *Streptopelia risoria* var. *alba*, with 74 chromosomes. 9. Karyotype of *Eulabeia indica*, with 72 chromosomes. 10. Karyotype of *Porphyrio poliocephalus viridis*, with 72 chromosomes. 11. Karyotype of *Ardea cinerea*, with 62 chromosomes.

Table 1. Summary of chromosome studies in fourteen species of birds.

Species	Modal chrom. number	Sex-chromosome	
		Z-chromosome	W-chromosome
<i>Struthio camelus camelus</i>	2n, 80	—	—
<i>Larus argentatus</i>	2n, 82	4th, meta.	9th, submeta.
<i>Turdus sibiricus davisoni</i>	2n, 80	—	—
<i>Oths scop japonicus</i>	2n, 76	—	—
<i>Syrnaticus reevesii</i>	2n, 82	—	—
<i>Geopelia cuneata</i>	2n, 72	3rd, subtelo.	7th, telo.
<i>Streptopelia risoria</i>	2n, 74	4th, meta.	8th, telo.
<i>Streptopelia risoria</i> var. <i>alba</i>	2n, 74	4th, meta.	8th, telo.
<i>Columba livia domestica</i>	2n, 80	4th, meta.	6th, meta
<i>Anser anser</i>	2n, 76	—	—
<i>Anser albifrons</i>	2n, 74	—	—
<i>Eulabeia indica</i>	2n, 72	—	—
<i>Ardea cinerea</i>	2n, 62	5th, submeta.	9th, submeta.
<i>Porphyrio poliocephalus viridis</i>	2n, 72	—	—

meta: metacentric, submeta: submetacentric, subtelo: subtelocentric, telo: telocentric.

same in morphological structure. Figure 9 represents a karyotype of *Eulabeia indica* with 72 chromosomes which comprise 14 macrochromosomes and 58 microchromosomes. Nos. 1, 2, 4 and 5 pairs are represented by submetacentric elements. No. 3 chromosomes are subtelocentric in appearance. Nos. 6 and 7 pairs are represented by telocentrics. At least 58 microchromosomes are determined in this metaphase plate.

The Malay gallinule (Porphyrio poliocephalus viridis)

The feather-pulp cultures from a sex unknown Malay gallinule provided 10 metaphases available for study. The chromosome number varied from 62 to 77. The chromosome complement showed 16 macrochromosomes and 46 to 51 microchromosomes. A karyotype with 72 chromosomes is presented in Figure 10. Nos. 1 to 5 chromosomes are submetacentric in general feature. They are well defined by size and centromere position. No. 6 pair is represented by submetacentric elements. Nos. 7 and 8 pairs are of telocentric structure. At least 56 microchromosomes are recognized in this plate.

The eastern grey heron (Ardea cinerea)

A karyotype study of *Ardea cinerea* was made by Yamashina (1950) in a male bird, reporting the diploid chromosome number of 76. The macrochromosomes were aligned into 6 pairs of meta- or submetacentrics and 2 pairs of telocentrics.

The present study was carried out in two birds of unknown sex, based on the feather-pulp cultures. The chromosome number showed a variation from 53 to 64 in 37 metaphases studied. The complement with 62 chromosomes (Fig. 11) consisted of 22 macrochromosomes (11 pairs) and 40 microchromosomes (20 pairs). The macrochromosomes were arranged into 8 pairs of meta- or submetacentric elements, and 3 pairs of

telo- or subtelocentrics. It seems most likely that the unpaired two chromosomes represent the sex chromosomes of this species, the larger one being the Z-chromosome, and the other the W-chromosomes.

SUMMARY

The morphology of chromosomes and the sex chromosome mechanism were studied in 14 species of birds, based on feather-pulp cultures, non-culture marrow cells. The results are summarized in Table 1.

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