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A Phylogenetic Study of Bird Karyotypes

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Abstract. Karyotypes were compared in 48 species, including 6 subspecies, of birds from 12 orders: *Casuariiformes, Rheiformes, Sphenisciformes, Pelecaniformes, Ciconiiformes, Anseriformes, Phoenicopteriformes, Gruiformes, Galliformes, Columbiformes, Falconiformes* and *Strigiformes.* — With the exception of the family *Accipitridae,* all the species studied are characterized by typical bird karyotypes with several pairs of macrochromosomes and a number of microchromosomes, though the boundary between the two is not necessarily sharp. The comparative study of complements revealed that a karyotype with 3 morphologically distinct pairs of chromosomes is frequently encountered in all orders except the *Strigiformes.* Those 3 pairs, submetacentric nos. 1 and 2, and a subtelocentric or telocentric no. 3, are not only morphologically alike but also have conspicuous homology revealed by the G-banding patterns. Furthermore, G-banding analysis provided evidence for the derivation of the owl karyotype from a typical bird karyotype.— The above cytogenetic features led to the assumption that the 3 pairs of marker chromosomes had been incorporated into an ancestral bird karyotype. It seems probable that those chromosomes have been transmitted without much structural changes from a common ancestor of birds and turtles, since the presence of the same marker chromosomes in the fresh water turtle *Geoclemys reevesii* is ascertained by G-banding patterns. — A profile of a primitive bird karyotype emerged through the present findings. Hence, it has become possible to elucidate mechanisms involved in certain structural changes of macrochromosomes observed in birds. It was concluded that a major role had been played by centric fission as well as fusion, translocation, and pericentric inversion.

Introduction

In the class *Aves*, karyotypes have so far been analysed in less than 2 percent of species, leaving about a half of the orders to be explored by means of current cytogenetic techniques. In spite of the paucity of data, a general resemblance of bird karyotypes is apparent. Examining the frequency distribution of chromosome numbers in 116 species, White (1973) pointed out that the birds have been extremely conservative as far as chromosome numbers are concerned.

Recently, the similarity of chromosome banding patterns in closely related species has successfully been utilized for the study of karyotype evolution in mammals (de Grouchy *et al.*, 1972; Evans *et al.*, 1973; Pathak *et al.*, 1973; Stock and Hsu, 1973; Yosida and Sagai, 1973). The banding homology, however, becomes indistinct in divergent

mammalian species, which may be due to either extensive rearrangements of chromosomes or alteration in the banding pattern *per se*, or both.

By the application of a G-banding technique to avian chromosomes, it appears possible to examine the extent of conservatism shown by the G-band itself in the long evolutional history on the one hand, and to provide confirmatory evidence for the homology of individual chromosomes or chromosome segments in divergent bird species on the other. The present data clearly showed that G-banding is exceedingly conservative; hence, analysis of karyotypic diversification is tenable between remotely related bird species. A model of the primitive bird karyotype emerged through the present comparative study helped understanding the mechanism of evolutionary changes in bird karyotypes.

Materials and Methods

We studied 48 species, including 6 subspecies, of birds belonging to 12 different orders as listed in Table 1. All specimens, except domestic fowls, were obtained from zoological gardens or breeding centers in Japan. The chromosome preparations were made from cultured peripheral lymphocytes (Takagi *et al.*, 1972). When the external sex was ambiguous the sex was determined on the basis of the sex chromosome constitution. Interspecific comparisons were made primarily on homogametic karyotypes. The length and the centromeric index in the 12 largest pairs of chromosomes were measured in 5 well-delineated metaphasic cells from a representative species of each order. The relative length (RL) of individual chromosomes was expressed as per cent of the total length of the haploid set excluding the chromosomes smaller than the 12th pair, since we could not be confident with measurements of minute elements. Errors introduced by the omission of small chromosomes may not be serious for the present purposes. For the convenience of karyotype comparison, chromosomes were tentatively divided into 3 size groups; large (A, $RL \geqq 10\%$), medium (B, $10\% > RL \geqq 6\%$), and small (C, $6\% > RL$). Each group was subdivided into 3 morphological categories; metacentric (m), submetacentric (sm), and subtelocentric (st)/telocentric (t). A serial numbering system according to the decreasing size was used to designate individual chromosomes in each species. G-banding patterns were obtained by a modification of the trypsin technique (Seabright, 1971). The optimal length of treatment by 0.2% trypsin in phosphate buffered saline (pH 7.0) varied from species to species.

Results

Tables 2 and 3 summarise the chromosomal findings. Excepting the family *Accipitridae*, the majority of species studied had 3 large pairs of morphologically corresponding chromosomes; submetacentric no. 1 and no. 2, and subtelocentric or telocentric no. 3. Adopting the term used by Bianchi *et al.* (1969) those chromosomes are referred as "shared" group A in the subsequent description. The number of group B chromosomes in homogametic sex varied from 2 to 10 with a sharp mode at 6. The precise number of group C chromosomes was usually difficult to

Table 1. List of species examined

Order	Family	Species	No. of specimens	
			♀	♂
Casuariiformes	*Casuariidae*	*Dromiceius novaehollandiae*	1	1
Rheiformes	*Rheidae*	*Rhea americana*	1	1
Sphenisciformes	*Spheniscidae*	*Spheniscus humboldti*	1	5
Pelecaniformes	*Pelecanidae*	*Pelecanus onocrotalus*	2	1
Ciconiiformes	*Ciconiidae*	*Ciconia ciconia ciconia*	1	1
		Ciconia ciconia boyciana	3	1
		Ephippiorhynchus senegalensis		1
		Leptoptilus crumeniferus		2
	Threskiornithidae	*Platalea leucorodia*	1	
		Nipponia nippon	1	1
		Threskiornis melanocephalus	3	4
		Threskiornis aethiopica		1
		Eudocimus ruber	1	2
Anseriformes	*Palamedae*	*Chauna chavaria*	1	3
	Anatidae	*Anser fabalis*	2	
		Branta canadensis	3	
		Cygnus olor		1
		Cygnus cygnus	2	
Phoenicopteriformes	*Phoenicopteridae*	*Phoenicopterus ruber*		3
Gruiformes	*Gruidae*	*Grus grus*	3	
		Grus japonensis	1	2
		Grus canadensis		1
		Grus vipio	2	4
		Grus antigone antigone	1	1
		Grus antigone sharpii	3	
		Anthropoides virgo		3
		Anthropoides paradisea	1	1
		Bugeranus carunculatus	2	3
Galliformes	*Cracidae*	*Balearica pavonia pavonia*	1	1
	Phasianidae	*Mitu mitu*		1
		Gallus domesticus	3	3
		Polyplectron bicalcaratum		1
Columbiformes	*Columbidae*	*Phasianus colchicus*		1
Falconiformes	*Cathartidae*	*Columba livia*		1
		Vultur gryphus	2	2
	Accipitridae	*Sarcorhamphus papa*	1	4
		Milvus migrans	1	1
		Pernis apivorus	1	
		Spizaetus nipalensis	1	
		Aquila heliaca		2
		Aquila chrysaetos		5
		Haliaetus leucocephalus		1
		Haliaetus pelagicus	1	2
		Pithecophaga jefferyi	1	
Strigiformes	*Strigidae*	*Sarcogyps calvus*	1	1
		Strix uralensis uralensis	2	1
		Strix uralensis japonica	1	
		Pulsatrix perspicillata		1

Table 2. Summary of karyotype analyses in 39 species of birds and a turtle

Species	2n	No. of chromosomes[a]								Sex chromosomes	
		A			B			C			
		m	sm	st/t	m	sm	st/t	m/sm	t	AN[b]	Z	W
D. novaehollandiae	80±		4	2			6		68±	68±	4–6th, t	4–6th, t
R. americana	82±		4	2		2	4		70±	70±	4–6th, ?	4–6th, ?
S. humboldti	78±		4	2	4	6		2	60±	72±	4th, sm	S[c], sm
P. onocrotalus	66		4	2	2	6		14	38	70	4th, sm	S, t
C. c. ciconia	68±		4	2	2	8		12	40±	72±	6th, m	S, st
C. c. boyciana	68		4	2	2	8		12	40	72	6th, m	S, st
E. senegalensis	68±		4	2	2	8		12	40±	72±	4–8th, ?	?
L. crumeniferus	72±		4	2	2	8		8	48±	72±	4–8th, ?	?
P. leucorodia	70±	2	4	4		6		10	44±	70±	6th, m	S, st
N. nippon	68	2	4	4		6		10	42	68	6th, m	S, st
T. melanocephalus	68	2	2	4		6	2	12	40	70	5th, m	S, sm
T. aethiopica	68±	2	6	2		6		10	42±	70±	6th, m	?
E. ruber	68±	2	6	2		6		10	42±	70±	6th, m	S, t
C. chavaria	80		4	2			6	2	66	70	5th, t	S, t
A. fabalis	80±		4	2	2	2	2		68±	68±	5th, sm	S, st
B. canadensis	80±		6	2			4		68±	68±	4th, sm	S, sm
C. olor	80±		4	4			4		68±	68±	4th, t	?
C. cygnus	80±		4	4			4		68±	68±	4th, t	S, t
P. ruber	80±		4	2		6		2	66±	70±	4–6th, sm	?
G. grus	80±		4	2		6		2	66±	70±	4th, sm	S, sm
G. japonensis	80±		4	2		6		2	66±	70±	4th, sm	S, sm
G. canadensis	80±		4	2		6		2	66±	70±	4–6th, sm	?
G. vipio	80		4	2		6		2	66	70	4th, sm	S, sm
G. a. antigone	80±		4	2		6		2	66±	70±	4th, sm	S, t
G. a. sharpii	80		4	2		6		2	66	70	4th, sm	S, t
A. virgo	80±		4	2		6		2	66±	70±	4–6th, sm	?
A. paradisea	80±		4	2		6		2	66±	70±	4th, sm	S, sm
B. carunculatus	80		4	2		6		2	66	70	4th, sm	S, sm
B. p. pavonia	80±		4	2		6		2	66±	70±	4th, sm	S, sm
M. mitu	82±		4	2		2	4		70±	70±	5th, m	?
G. domesticus	78	2	6	2			2	4	62	70	5th, m	S, m
P. bicalcaratum	78±	2	6	2			2	4	62±	70±	5th, m	?
P. colchicus	82±		4	4			8		66±	68±	4th, sm	?
C. livia	80±		4	2	2	4			68±	68±	4th, m	?
V. gryphus	80		4	2		6		2	66	70	5th, sm	S, m
S. papa	80±		4	2		6		2	66±	70±	5th, sm	S, m
S. u. uralensis	82±	2	4	6			2		68±	70±	5th, m	S, m
S. u. japonica	82	2	4	6			2		68	70	5th, m	M[d], m
P. perspicillata	76±	4	2	2	4			4	60±	68±	5–6th, m	
G. reevesii	52		4	2	2	4	8	24	8	64–68	?	?

[a] In homozygous sex including two Z chromosomes.
[b] Corrected number of arms (see text).
[c] Small.
[d] Medium-sized.

determine, but the counts appeared quite convincing in some excellent plates. Chromosomes of this group varied from 52 to 70 in different species. Since the chromosomes of 9 species of the *Accipitridae* showed a gradual seriation in size, they were not specifically classified into the above 3 groups (Table 3, p. 102). Brief accounts of karyotypes in each order are given below.

Casuariiformes

The karyotype of *D. novaehollandiae* is identical with that we reported previously (Takagi *et al.*, 1972). In short, group A is the "shared" type. Group B consists of 3 pairs of acrocentrics. A conspicuous secondary constriction is apparent at the proximal end of the long arm in one of those pairs. Group C includes about 68 small acrocentrics. Heteromorphic sex chromosomes are not present in both sexes.

Rheiformes

R. americana has a karyotype very similar to that of *D. novaehollandiae*. The centromere locates more distally in no. 2 chromosomes of this species. Unlike *D. novaehollandiae*, a pair of group B is submetacentric and no conspicuous secondary constriction is detected in any B-group chromosome. Sex chromosomes are not identified with certainty.

Sphenisciformes

Group A is the "shared" type in *S. humboldti* (Fig. 1). Five pairs of metacentric or submetacentric chromosomes are comprised in group B. Group C is composed of 30 pairs of telocentrics and a pair of metacentrics. The Z is a medium-sized submetacentric and the W a small submetacentric.

Pelecaniformes

P. onocrotalus has the "shared" type group A (Fig. 2). Group B includes a pair of metacentrics and 3 pairs of submetacentrics. Group C consists of a total of 52 chromosomes, 14 of which are metacentric or submetacentric. The Z is a medium-sized submetacentric and the W a small telocentric.

Ciconiiformes

The "shared" type group A is found in 4 out of 9 species under study, *C. c. boyciana*, *C. c. ciconia*, *E. senegalensis*, and *L. crumeniferus*. Out of 52–56 C group elements 8–12 are apparently biarmed. Group B of those species consists of 5 pairs of metacentric or submetacentric elements. The Z is a medium-sized metacentric and the W a small subtelocentric.

Fig. 1

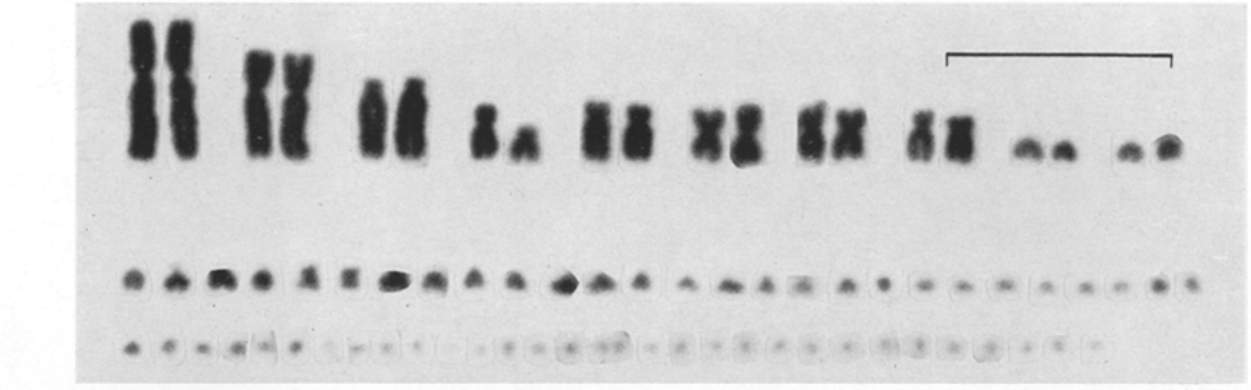

Fig. 2

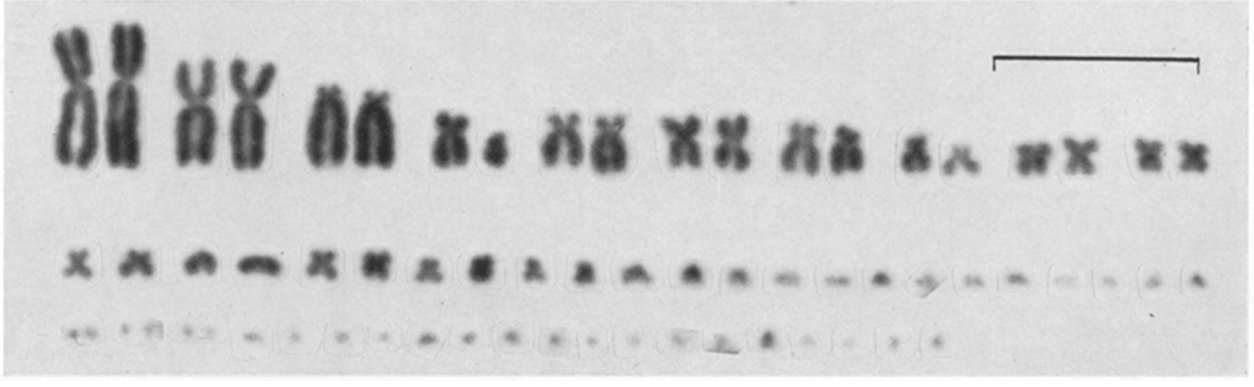

Fig. 1.[1] ♀ karyotype of the Humboldt penguin (*Spheniscus humboldti*, 2n= 78±). Sex chromosomes at the 4th position
Fig. 2. ♀ karyotype of the white pelican (*Pelecanus onocrotalus*, 2n=66), Sex chromosomes at the 4th position

1 The bars in Figs. 1—8 represent 10 μm.

Instead of no. 1 of the "shared" group A, a telocentric pair similar in length to no. 3 is present in *T. melanocephalus* (Fig. 3). Group B consists of 3 pairs of submetacentric and a pair of telocentric elements. The combined relative lengths of the two telocentric elements of groups A and B closely agree with that of no. 1 of *C. c. boyciana* (Table 5, p. 110). Furthermore, a large submetacentric and a small metacentric pair in *T. melanocephalus* have made appearance at the expense of no. 6 and no. 8 submetacentrics of *C. c. boyciana*. The Z is similar to that of the above 4 species, while the W is a small submetacentric.

The karyotypes of *N. nippon* and *P. leucorodia* are almost identical and are similar to the karyotype of *T. melanocephalus* (Fig. 14, p. 113). The only difference is the appearance of an extra submetacentric pair in group A in place of 2 telocentric pairs of groups B and C of *T. melanocephalus*.

T. aethiopica and *E. ruber* possess an identical karyotype which is similar to that of *N. nippon* and *P. leucorodia*. A pair of telocentrics in group A of the latter 2 species is substituted by a pair of subtelocentrics of comparable size in the former two.

Anseriformes

Five species so far studied share a diploid chromosome number of $80 \pm$. Group A is the "shared" type. The Z is as long as no. 3 in *C. olor*, *C. cygnus* and *B. canadensis*, and it is assigned to group A in Table 2. Consequently, group B consists of 2 pairs of autosomes in those species, while it consists of 3 pairs including the Z in *C. chavaria* (Fig. 4) and *A. fabalis*. All elements of group C appear telocentric in every species. The W is small and varies from telocentric to submetacentric in different species.

Phoenicopteriformes

Group A is "shared" type in *P. ruber* (Fig. 5). Group B consists of 3 pairs of submetacentric elements. Two of 68 C chromosomes are bi-armed. Since only the homogametic sex was available for the present study, sex chromosomes were not identified.

Gruiformes

Eleven species of cranes studied have a diploid chromosome number of $80 \pm$ and their karyotypes (Fig. 6) are almost identical with that of *P. ruber*. The Z is a medium-sized submetacentric. The only karyotypic difference lies in the morphology of the W chromosome. It is telocentric in *G. a. antigone* and *G. a sharpii*, whereas it is submetacentric in *G. grus*, *G. japonensis*, *G. vipio*, *A. paradisea*, *B. carunculatus*, and *B. p. pavonia*. Sex chromosomes were not identified in *G. canadensis* and *A. virgo* since chromosomes were studied only in homogametic sex.

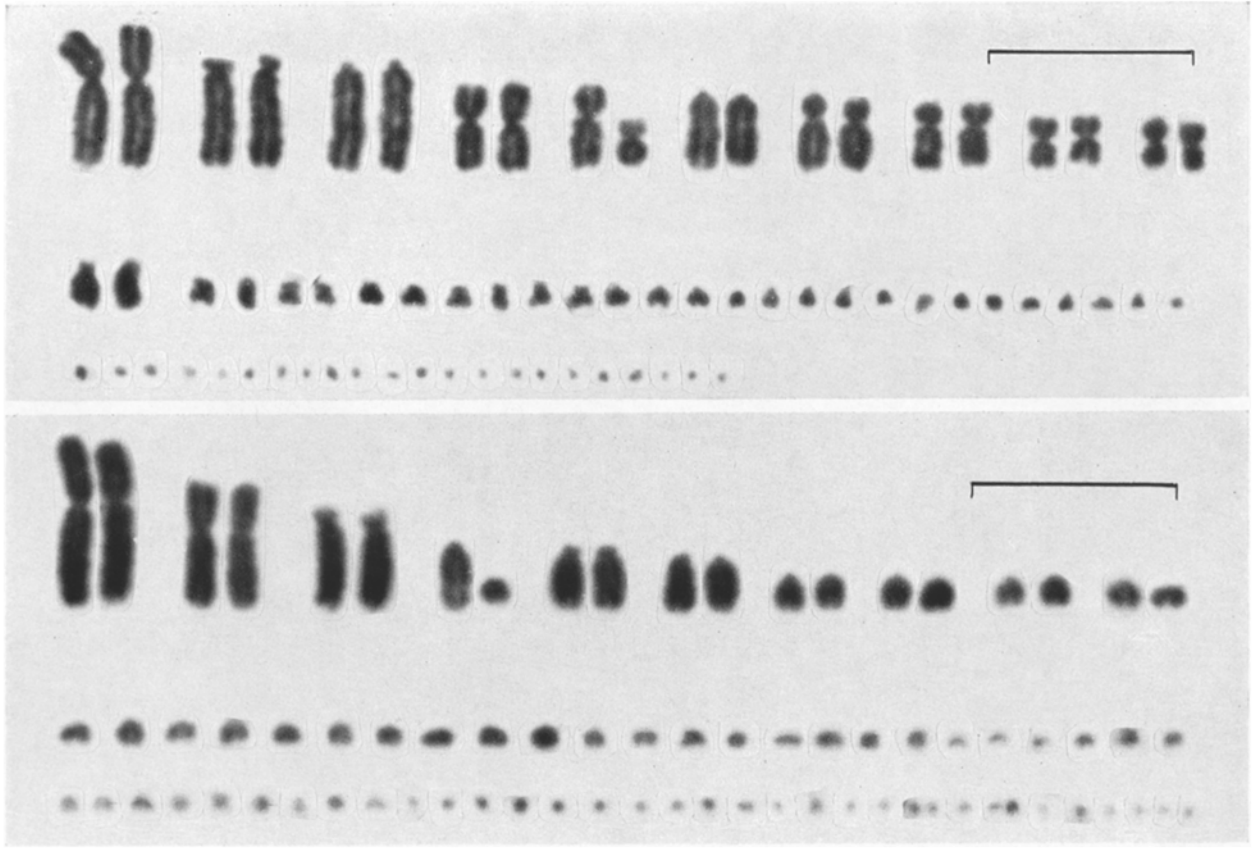

Fig. 3

Fig. 4

Fig. 3. ♀ karyotype of the black headed ibis (*Threskiornis melanocephalus*, 2n=68±). Sex chromosomes at the 5th position
Fig. 4. ♀ karyotype of the screamer (*Chauna chavaria*, 2n=80±). The telocentric Z, which shows a secondary constriction at the distal two-thirds, and the W at the 4th position

Galliformes

The karyotype of *M. mitu* is characterized by the "shared" type group A. Group B consists of 3 pairs of chromosomes one being submetacentric and the remaining 2 telocentric. The submetacentric element has been identified as the Z (Beçak *et al.*, 1971). At most 35 telocentric pairs are present in group C.

The karyotype of *P. bicalcaratum* is identical with that of *G. domesticus* reported by Owen (1965). In addition to the 3 pairs of "shared" elements, the submetacentric Z and a subtelocentric autosomal pair which are as long as no. 3 are included in group A. Consequently, group B consists of only one telocentric pair. The combined relative lengths of this telocentric pair and the subtelocentric pair classified into group A roughly correspond to the sum of the relative lengths of no. 5 and no. 6 of *M. mitu*.

The karyotype of *P. colchicus* is identical with that reported earlier (Takagi and Makino, 1966). In short, except for the largest submetacentric pair, all the other autosomes are telocentric. The loss of no. 2 of the "shared" group A coincides with the gain of 2 pairs of telocentric elements, one being in group A and the other in B. The submetacentric Z is comparable in length to no. 3.

Columbiformes

The karyotype of *C. livia* is identical with that reported earlier (Galton and Bredbury, 1966), showing 3 pairs of "shared" elements in group A and 3 biarmed pairs in group B. All the C group chromosomes are telocentric.

Falconiformes

The karyotypes of *V. gryphus* and *S. papa* (Fig. 7) are nearly identical with each other and with those of *P. ruber* as well as all members in the family *Gruidae*. The Z is a medium-sized submetacentric element and the W a small metacentric.

The remaining 9 species of the family *Accipitridae*, as the family *Falconidae* reported by Renzoni and Vegni-Talluri (1966), have markedly different karyotypes not only from species of other orders but also from those of the allied family *Cathartidae*. Fig. 8 shows a representative karyotype of *M. migrans* illustrating the extent of diversification of karyotype in this family. Table 3 summarizes karyotypic findings in the 9 species. Except for 6–10 minute elements, individual chromosomes are considerably larger than usual micro-chromosomes of other species. The Z so far identified, is the largest or the second largest element in the complement. If this chromosome is comparable in size to the Z of

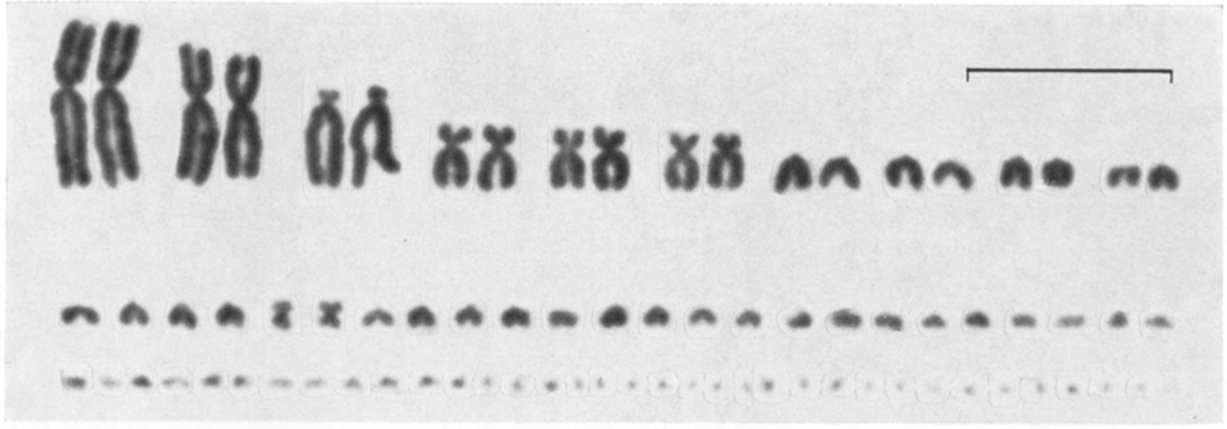

Fig. 5

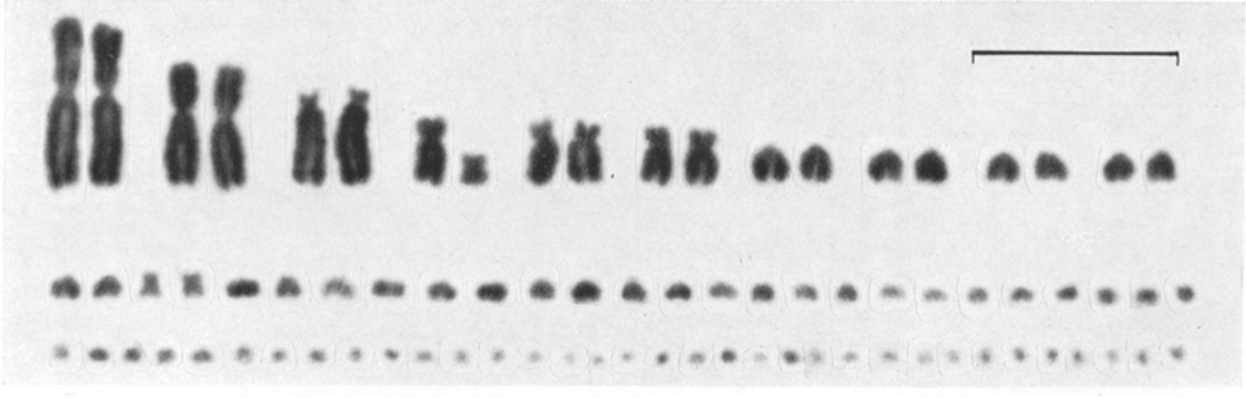

Fig. 6

Fig. 5. ♂ karyotype of the American flamingo (*Phoenicopterus ruber*, 2n=80). The Z is unidentified
Fig. 6. ♀ karyotype of the wattled crane (*Bugeranus carunculatus*, 2n=80 ±). Sex chromosomes at the 4th position

Fig. 7

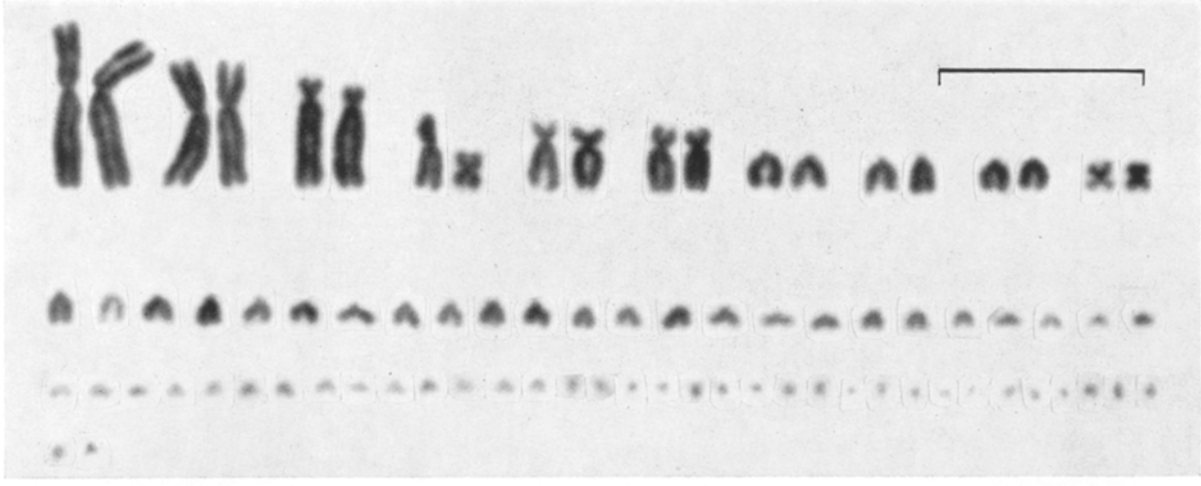

Fig. 8

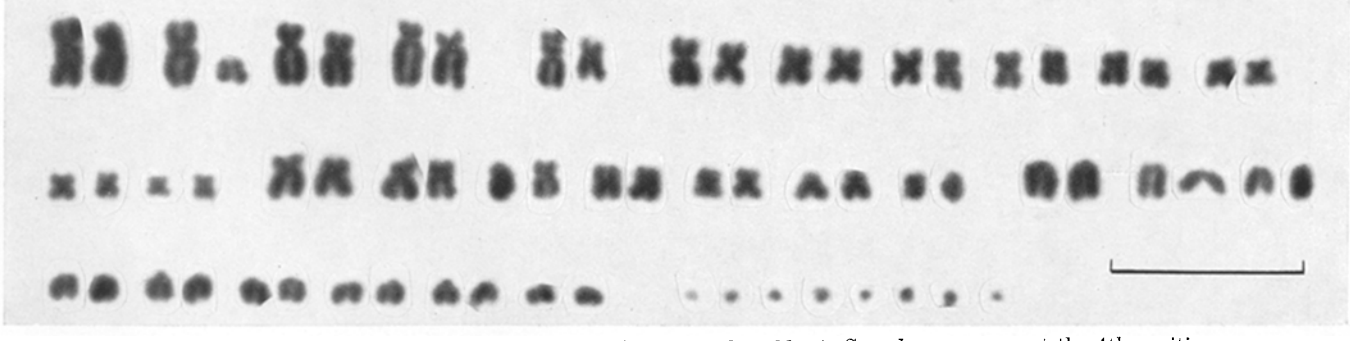

Fig. 7. ♀ karyotype of the king vulture (*Sarcorhamphus papa*, 2n=80±). Sex chromosomes at the 4th position
Fig. 8. ♀ karyotype of the black-eared kite (*Milvus migrans*, 2n=66). Sex chromosomes at the 2nd position

Table 3. Summary of karyotype analyses in 9 species of the *Accipitridae*

Species	2n	Number of chromosomes					Sex chromosomes	
		m	sm	st	t	minute	Z	W
M. migrans	66	18	16	6	18	8	2nd, sm	S, sm
P. apivorus	66	18	12	10	20	6	1st, sm	M, st
S. nipalensis	68	10	16	6	28	8	1st, sm	M, t
A. heliaca	68	14	8	10	26	10	?	?
A. chrysaetos	62	18	14	10	14	6	?	?
H. leucocephala	66	16	10	12	20	8	?	?
H. pelagicus	66	18	10	12	18	8	1st, sm	S, st
P. jefferyi	66	12	10	10	26	8	1st, sm	S, st
S. calvus	68	12	12	10	24	10	1st, sm	S, st

typical avian karyotypes (Ohno, 1967), it appears that most of the C elements have gained in length at the expense of group A chromosomes. A medium-sized subtelocentric pair of the majority of species in this family possesses prominent satellite bodies at the distal end of the short arm, which frequently show an end-to-end association.

Strigiformes

The karyotypes of *S. u. uralensis* and *S. u. japonica* are nearly identical with each other and are similar to those of *S. alco* (Hammer, 1970) and *Bubo v. virginianus* (Krishan *et al.*, 1966). Group A consists of 3 pairs of acrocentrics, 2 pairs of submetacentrics and a pair of metacentrics. The rest of the complement, a pair of group B and 34 pairs of group C, are telocentric. The metacentric element assigned to group A is the Z. The metacentric W is small in *S. u. uralensis*, while it is medium-sized in *S. u. japonica*. A pair of large metacentrics is conspicuous in *P. perspicillata*. The size of this chromosome corresponds to the sum of group A telocentrics in the above 2 species.

Chromosome Measurements in Different Orders

Fig. 9 shows serial alignments of the 12 largest pairs of avian species representing 12 different orders and a species of turtle representing the reptilian order *Chelonia*. The most striking feature is the good agreement in size and centromeric position of the 3 largest pairs in both

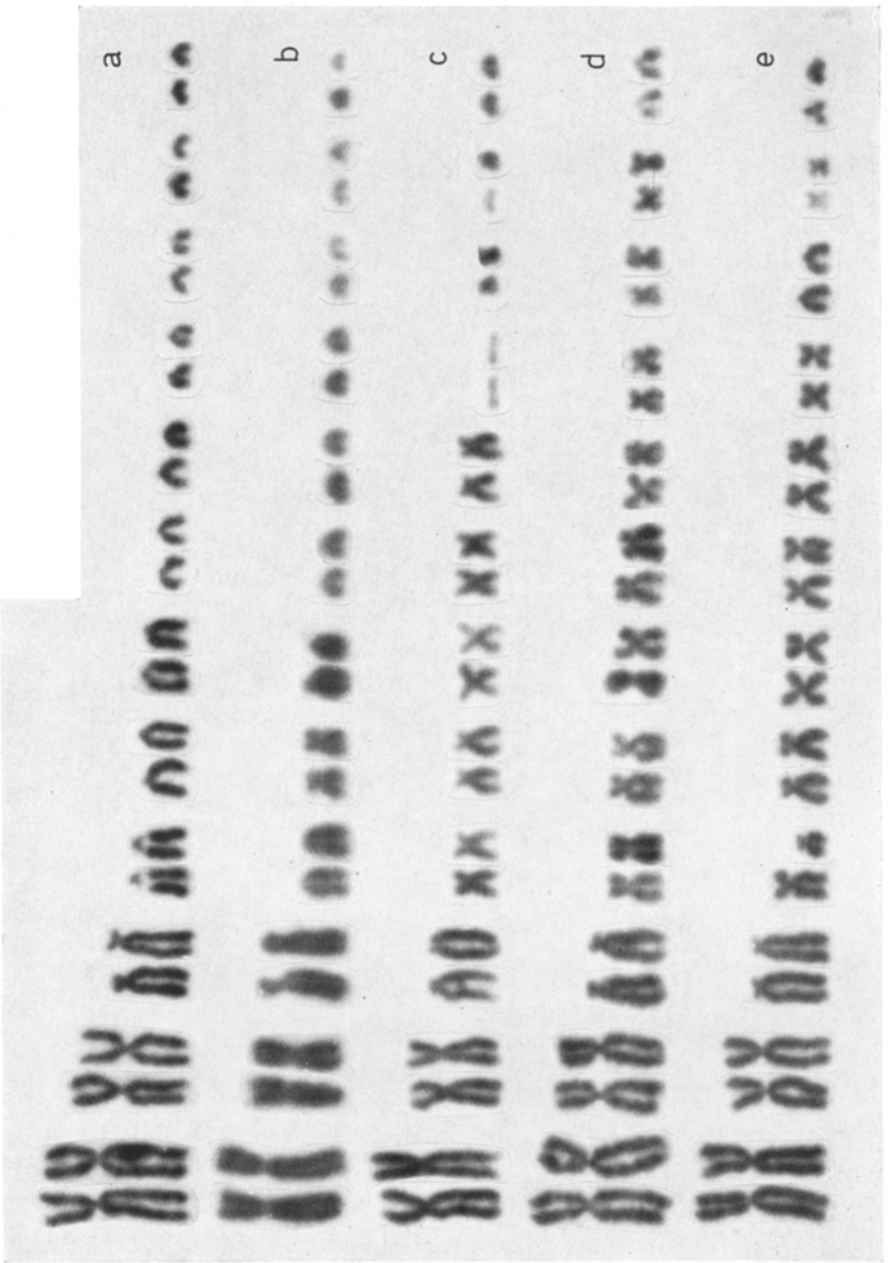

Fig. 9a—e

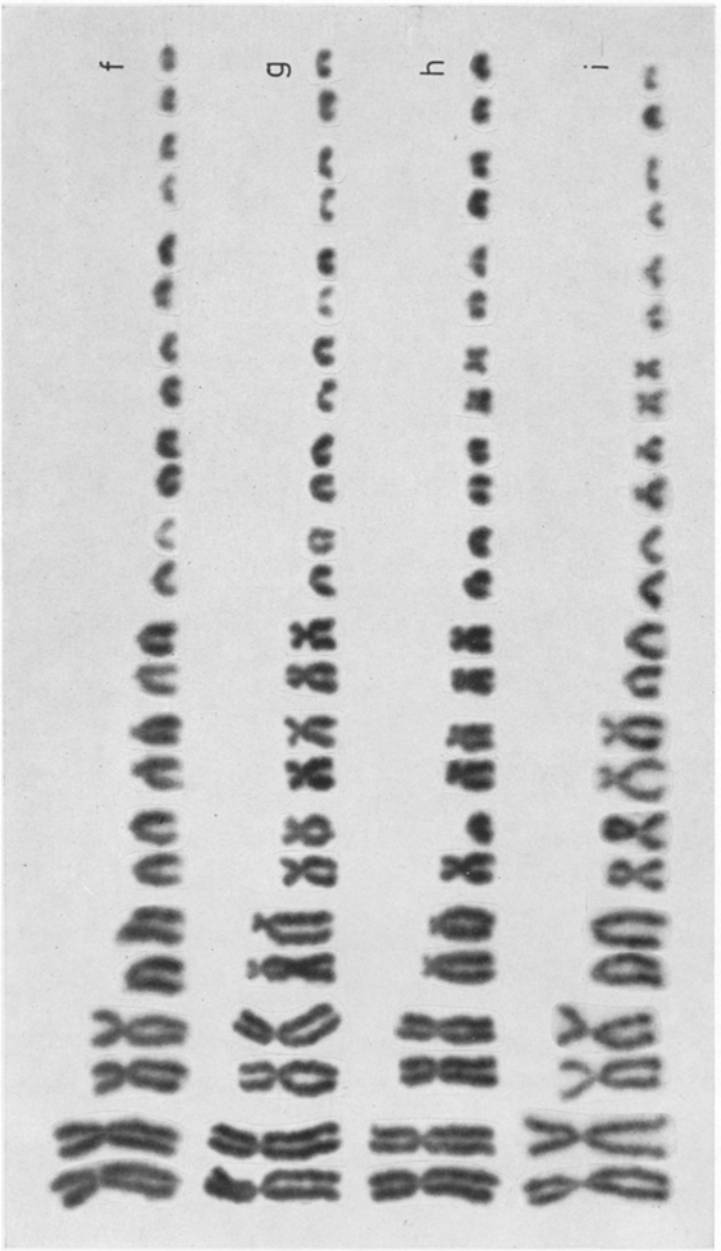

Fig. 9f—i

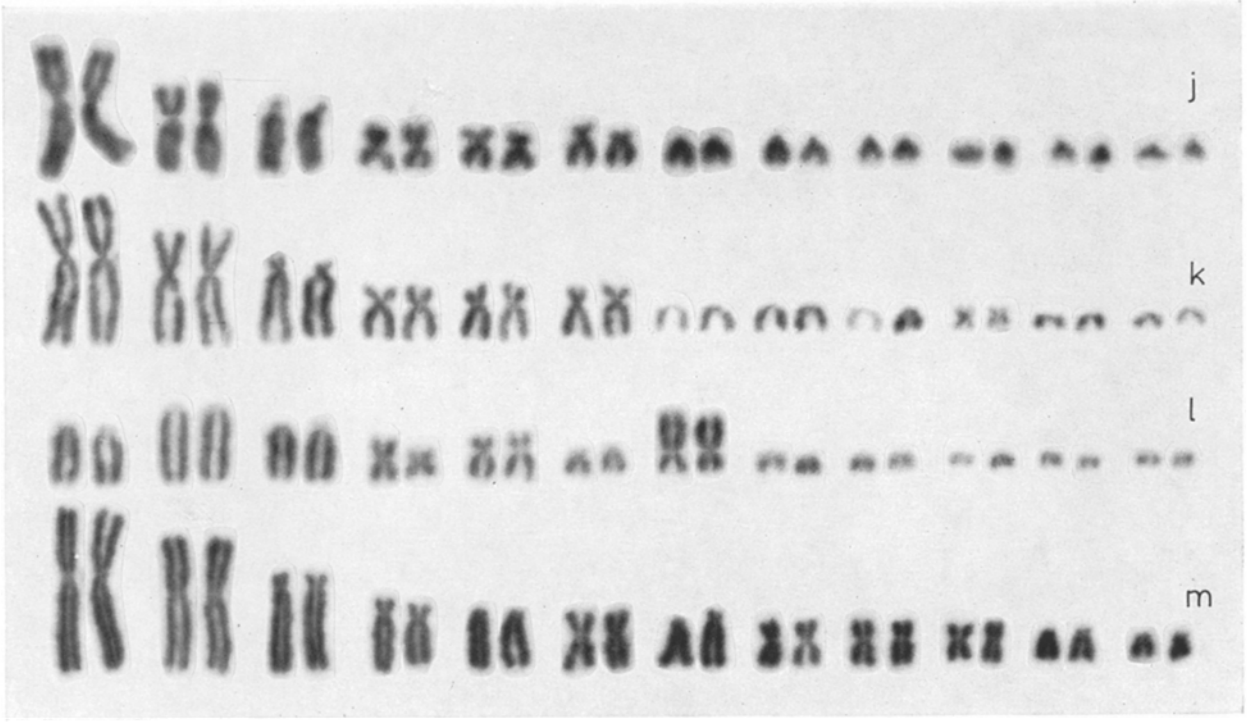

Fig. 9j—m

Fig. 9a—m. Partial karyotypes of a representative species from 12 avian and 1 reptilian orders. (a) Emu, *D. novaehollandiae*, (b) rhea, *R. americana*, (c) Humboldt penguin, *S. humboldti*, (d) white pelican, *P. onocrotalus*, (e) eastern white stork, *C. c. boyciana*, (f) screamer, *C. chavaria*, (g) American flamingo, *P. ruber*, (h) Lurmese sarus crane, *G. a. sharpii*, (i) domestic chicken, *G. domesticus*, (j) pigeon, *Columba livia*, (k) condor, *V. gryphus*, (l) Ural owl, *S. u. japonica*, (m) fresh water turtle, *G. reevesii*. Magnification varies among species

Table 4. Relative lengths (top) and centromeric indices

Species	Pairs of chromosomes				
	1	2	3	Z	W
Dromiceius	23.4	17.3	13.6	9.3[a]	—
novaehollandiae	39.7	44.6	16.0	0	
Rhea	23.0	16.4	14.9	8.3[a]	—
americana	36.5	43.5	20.4	0	
Spheniscus	20.9	15.1	12.3	8.3	5.0
humboldti	39.4	38.0	16.3	37.2	34.2
Pelecanus	19.6	15.2	11.5	8.5	3.0
onocrotalus	38.9	39.4	17.6	36.5	0
Ciconia	19.4	15.2	11.4	7.1	4.5
c. boyciana	41.7	38.3	14.6	45.0	27.7
Chauna	22.7	17.6	13.4	8.4	3.2
chavaria	39.8	37.2	17.7	0	0
Phoenicopterus	21.5	17.0	13.3	9.0[a]	—
ruber	39.4	39.1	15.9	36.6	
Grus	20.7	16.1	12.6	9.4	4.5
antigone sharpii	40.0	38.9	17.4	35.2	0
Gallus	23.4	17.5	12.2	10.0	3.8
domesticus	37.9	36.2	0	47.8	40.3
Columba	22.4	16.4	12.7	8.4	—
livia	41.0	40.5	15.9	47.2	
Vultur	20.7	15.8	13.4	8.6	5.2
gryphus	40.2	38.6	19.8	36.8	47.2

[a] Identification is tentative.

avian and reptilian species, with the exception of *S. u. uralensis* of the order *Strigiformes*. The number and morphology of B-group chromosomes, including the Z pair, on the other hand, vary considerably in different species.

Table 4 summarizes results of chromosome measurements in the 11 avian species shown in Fig. 9. The results substantiate the visual impression that the 3 largest pairs are shared by those species. In fact, the mean relative length of submetacentric no. 1 is 21.4 with a range from 19.4 to 23.4. The centromere index varies from 36.5 to 41.7 with a mean of 39.5. No. 2 appears almost identical in all the species except for *D. novaehollandiae*, *R. americana*, and *G. domesticus*. The centromere index is much larger in the former 2 species (44.6 and 43.5), while it is

(bottom) of 12 largest pairs from 11 diverse species

5	6	7	8	9	10	11	12
7.9	7.0	5.0	4.3	3.8	3.4	2.9	2.7
0	0	0	0	0	0	0	0
7.8	7.3	4.4	4.1	3.8	3.6	3.3	3.2
41.5	0	0	0	0	0	0	0
8.3	8.1	7.4	7.3	3.4	3.2	3.0	2.8
31.7	45.7	43.9	39.5	0	0	0	0
7.7	7.4	6.7	5.2	4.9	4.7	4.3	4.0
29.6	47.8	34.5	47.4	37.7	46.3	44.4	24.4
8.2	8.2	7.1	7.0	5.2	4.5	3.6	3.1
36.5	31.6	39.2	46.1	47.8	0	47.2	0
8.9	7.4	4.7	4.2	3.8	3.3	3.0	2.7
17.6	0	0	0	0	0	0	0
8.4	8.0	4.7	4.5	4.2	3.8	3.2	3.0
34.7	34.0	0	0	0	0	0	0
8.5	7.6	5.2	4.9	4.3	4.0	3.6	3.4
29.2	34.2	0	0	41.7	0	0	0
10.5	6.2	4.4	3.8	3.4	3.2	2.8	2.7
25.9	0	0	29.8	40.2	0	0	0
7.9	7.6	5.0	4.8	4.4	3.9	3.6	3.1
38.8	33.3	0	0	0	0	0	0
8.8	8.1	5.1	4.2	4.1	3.7	3.6	2.9
33.2	34.4	0	0	47.6	0	0	0

smaller in the last species (36.2), as compared to the mean value (38.8) of the remaining 8 species. With the exception of *G. domesticus*, the pair no. 3 is subtelocentric. Considering the morphological similarity and a rather narrow range of the relative lengths (11.4 to 14.9), no. 3 chromosomes of all the species appear to correspond each other.

As described earlier, the number and morphology of B group chromosomes are variable in different orders. However, the relative lengths of pairs located at the corresponding position in the karyotype are similar in *D. novaehollandiae*, *C. chavaria*, *P. ruber*, *V. gryphus*, *C. livia* and *G. a. sharpii*, although the centromeric indices are variable. The lengths of larger C group chromosomes also correspond well in those species. In contrast, the Z is rather variable in both length (7.1–10.0) and centromeric index (0–47.8). Hence the Z is assigned to either

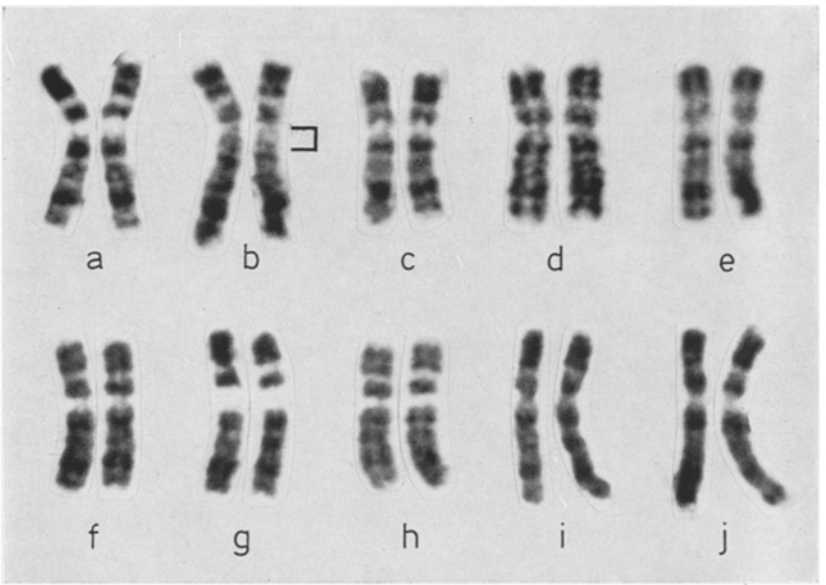

Fig. 10a—j. G-banding patterns of no. 1 chromosomes from 9 bird and 1 chelonian species. (a) *D. novaehollandiae*, (b) *R. americana*, (c) *P. onocrotalus*, (d) *C. c. boyciana*, (e) *C. chavaria*, (f) *P. ruber*, (g) *V. gryphus*, (h) *G. a. sharpii*, (i) *G. domesticus*, (j) *G. reevesii*. Magnification varies among species

group A or B. The W chromosome also varies considerably in size and morphology.

G-Banding Analysis

The above morphological studies may suggest but not assure that morphologically corresponding chromosomes in the diverse species are actually homologous. With a hope to provide more critical data, G-banding patterns were analysed in 11 species from 9 avian orders and in 1 chelonian species. Due to technical difficulty to produce adequate banding patterns, the comparison was restricted to group A chromosomes in most species.

Fig. 10 shows no. 1 pairs from 9 avian and 1 reptilian species. It is obvious that these banding patterns are essentially identical in those 10 species. The only exception is an extra segment at the proximal end of the long arm in *R. americana*.

Banding patterns in no. 2 are less distinct than in no. 1. No. 2 of *D. novaehollandiae* and *R. americana* lack a segment at the proximal end

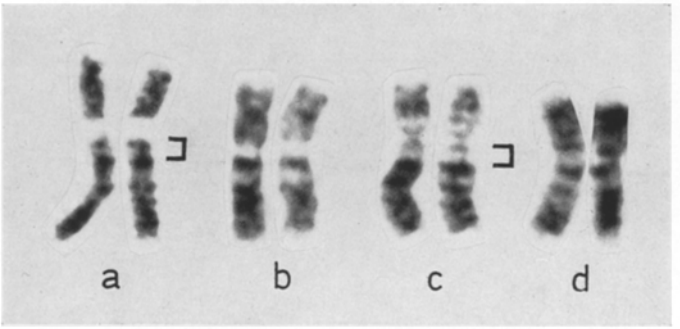

Fig. 11a—d. G-banding patterns of no. 2 chromosomes from 4 species of birds. (a) *V. gryphus*, (b) *D. novaehollandiae*, (c) *G. domesticus*, (d) *R. americana*. The chromosomes of *D. novaehollandiae* and *R. americana* lack the segment corresponding the one marked on the chromosomes of *V. gryphus* and *G. domesticus*. Magnification varies among species

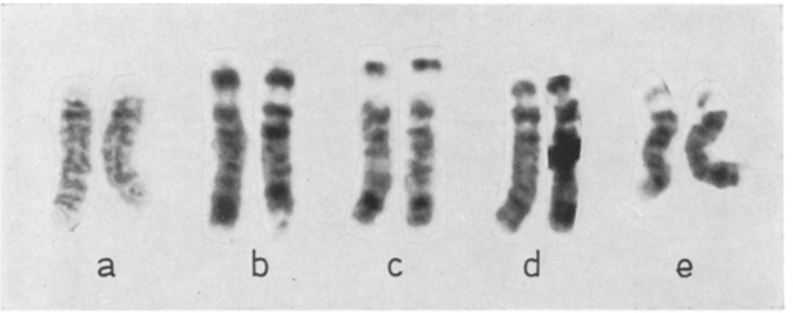

Fig. 12a—e. G-banding patterns of no. 3 chromosomes from 5 species of birds. (a) *G. domesticus*, (b) *R. americana*, (c) *V. gryphus*, (d) *P. ruber*, (e) *G. a. sharpii*. Magnification varies among species

of the long arm (Fig. 11). Otherwise, banding patterns of this pair are comparable in all the species. The absence of the above mentioned segment is responsible for the disproportionately large centromere indices in the ratite species.

A limited number of metaphases with banded patterns suitable for comparison unequivocally indicates that no. 3 chromosomes are comparable from species to species (Fig. 12). Banding patterns of no. 3 telocentrics of *G. domesticus* correspond to the long arm of no. 3 subtelocentrics of the other species.

The karyotype of *S. u. uralensis* appears to differ considerably from the common avian karyotypes having "shared" group A. Nevertheless,

Table 5. Presumptive karyotypic relationships between *T. melanocephalus*, *P. leucorodia* and *C. c. boyciana*

	Chromosomes of		Counterparts in *C. c. boyciana*	
	T. melanocephalus	*P. leucorodia*		
1	11.4	11.4	1q	11.4
2	14.8	15.6	2	15.2
	5.8	6.2		5.8
	9.1	9.3		9.4
3	11.6	11.6	3	11.4
	2.0	1.6		1.7
	9.9	10.1		9.8
Z	8.3	8.6	Z	7.1
	3.3	3.3		3.2
	5.0	5.3		3.9
W	4.9	4.6	W	4.5
	2.1	1.4		1.2
	2.8	3.2		3.2
5	7.3	11.8		
	0	3.9	10	4.5
	7.3	7.9	1p	8.1
6	9.4	9.4		
	3.9	4.2	8q	3.8
	5.5	5.3	6q	5.6
7	7.6	8.1	5	8.2
	2.6	2.3		3.0
	5.0	5.8		5.2
8	7.0	7.5	7	7.1
	2.6	2.4		2.8
	4.4	5.1		4.3
9	5.5	5.8		
	2.4	2.7	6p	2.6
	3.1	3.1	8p	3.2
10	5.3	5.4	9	5.2
	2.6	2.5		2.5
	2.7	2.8		2.7
11	5.1		10	4.5
	1.5			0
	3.6			4.5

p and q designate a short arm and a long arm, respectively.

Table 6. Presumptive karyotypic relationships between *S. u. japonica* and *G. domesticus*

	Chromosomes of *S. u. japonica*		Counterparts in *G. domesticus*	
1	13.3	1q	13.3
	1.8		0
	11.5		13.3
2	16.3	2	17.5
	1.4		6.3
	15.0		11.2
3	13.7	3	12.2
	2.2		0
	11.6		12.2
4	13.4		
	5.1	?	
	8.4	1p	8.9
Z	10.1	Z	10.0
	4.9		4.7
	5.2		5.2
W	6.5	W	3.8
	2.9		1.5
	3.5		2.3
6	9.5	5	10.5
	3.2		2.7
	6.3		7.8
7	6.0	6	6.2
	1.4		0
	4.6		6.2

the derivation of the owl karyotype from the latter is clarified by comparing relative lengths (Table 6) and G-banding patterns (Fig. 13). The relative length of the longest telocentrics of the owl corresponds to that of no. 2 of the chicken, suggesting a pericentric inversion has taken place. The comparison of G-banding patterns favors this interpretation. Two acrocentric pairs morphologically similar to no. 3 of the chicken complement have distinctively different banding patterns. One is similar to the pattern of chicken no. 3 and the other to that of the long arm of no. 1. The long arm of the longest submetacentric pair of the owl exactly corresponds to the short arm of chicken no. 1.

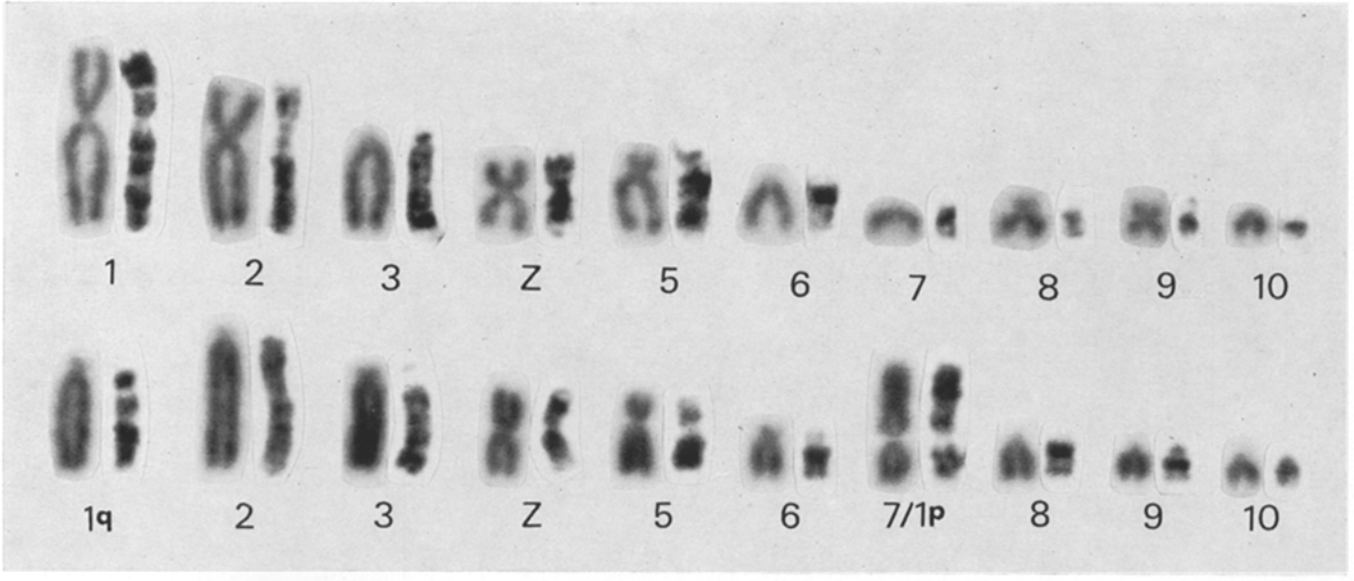

Fig. 13. Comparison of karyotypes between the domestic chicken, *G. domesticus* (top) and the Ural owl, *S. u. japonica* (bottom). Magnification varies between species

Fig. 14a—d. Karyotype changes observed in species from the family *Ciconiidae* and *Threskiornithidae*. (a) Eastern white stork *C. c. boyciana*, (b) black headed ibis, *T. melanocephalus*, (c) Japanese crested ibis, *N. nippon*, (d) scarlet ibis, *E. ruber*. Magnification varies among species

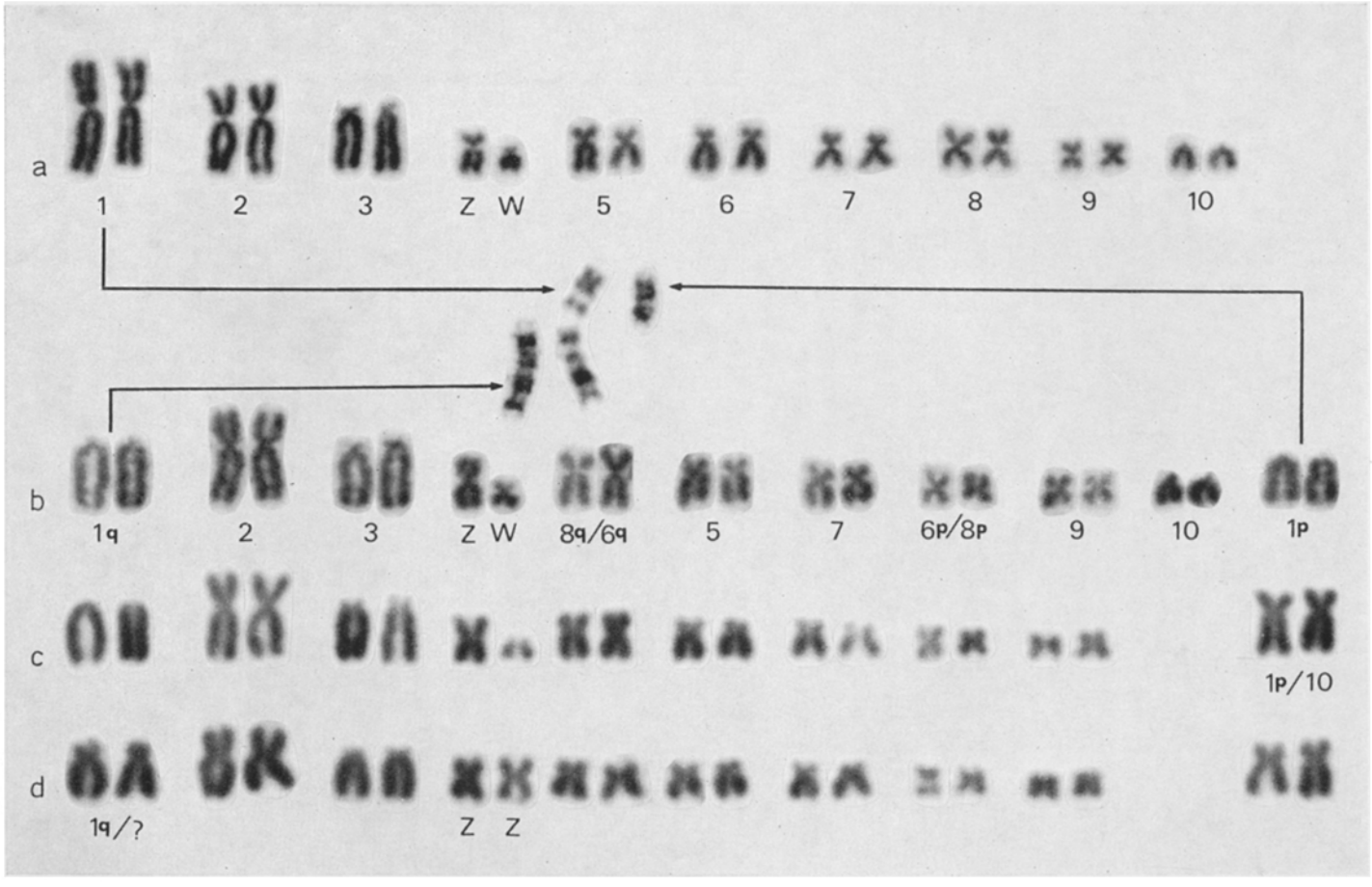

Fig. 14a—d

Discussion

The similarity of avian karyotypes was advocated by Ohno and collaborators on the basis of direct karyotype analyses (Stenius *et al.*, 1963; Ohno *et al.*, 1964) and the uniform genome size in diverse orders of bird (Atkins *et al.*, 1965; Bachmann *et al.*, 1972). Hammer (1966, 1970) unequivocally demonstrated that closely related bird species often have identical karyotype though exceptions are not rare (Ray-Chaudhuri *et al.*, 1969). Analysing then available data, Bloom (1969) showed that a mode of the diploid chromosome number was 80 in 91 avian species. The range was 52 to 94 with 77 per cent of the chromosome numbers in the 76 to 84 range.

On the other hand, considerable karyotypic variation in mammals led Hsu and Mead (1969) to mention that comparison between distantly related taxa may be meaningless. In most cases, comparing karyotypes of different genera within a family or subfamily, and sometimes even different species within a genus, can be futile because the chromosomes of these taxa bear no resemblance to one another. In sharp contrast, the remarkable conservatism of avian karyotypes has allowed chromosome comparison even between different orders. A profile of a primitive avian karyotype has thus emerged through the present comparative study.

A Primitive Avian Karyotype

The 3 macrochromosomal pairs of the "shared" group A occurs in 14 relatively primitive avian orders. Their homology was ascertained by almost identical G-banding patterns in 9 different orders. It should not be far-fetched to extrapolate this finding to the morphologically corresponding chromosomes found in species of the remaining 5 orders where G-baning studies have not yet been carried out. This conspicuous situation could not have been achieved without postulating a strong preferential selection pressure upon the karyotype itself and/or a mechanism evoking identical chromosomal rearrangements repeatedly, both of which appear quite improbable in evolution. Therefore, the most plausible conclusion appears that the 3 pairs of chromosomes have been transmitted from a common ancestor. The 3 largest pairs of chromosomes in the fresh water turtle, *Geoclemys reevesii* (Sasaki and Itoh, 1967), appear homologous to the avian counterparts, in view of their nearly identical banding patterns. This suggests that the incorporation of these marker chromosomes into the turtle-bird genome lineage is dated back to considerably earlier era than the advent of an ancestral bird.

In spite of the conspicuous conservation of the group A complement, considerable variability in number and morphology of the remaining elements prevents from reconstructing the ancestral pattern with certainty. Group B consists of 3 pairs in homogametic sex in 8 diverse orders, *Casuariiformes, Rheiformes, Anseriformes, Phoenicopteriformes, Galliformes, Gruiformes, Falconiformes* and *Columbiformes*, while it comprise 4 or 5 pairs in 5 orders, *Sphenisciformes, Colymbiformes* (Hammer, 1970), *Charadriiformes* (Hammer, 1966, 1970; Itoh *et al.*, 1969), *Pelecaniformes* and *Ciconiiformes*. Though final decision must await banding analysis, it appears that the 3 B-chromosome pairs in the former group are in fact homologous. Pericentric inversions may account differences in centromeric position of the constituent chromosomes. There is no indication that the former 8 orders represent one phylogenetic lineage and the latter 5 represent another. Actually some members of the former have closer relationship to some of the latter. Consequently, the simplest explanation appears that the group B consisting of 3 pairs of chromosomes has been transmitted from a common ancestor of both groups of birds. It is hard to maintain that the 3-pair pattern has evolved independently from some other patterns in different orders.

The diploid chromosome number is in the 60's in the order *Pelecaniformes, Ciconiiformes* and *Charadriiformes* (Hammer, 1966, 1970; Itoh *et al.*, 1969), while it is near 80 in most other orders. Obviously, the low diploid numbers coincide with the increase in the number of biarmed elements in group C. The total arm number of group C elements varies from 62 to 70 in all the species under study except the accipiter. The range becomes 68 to 72 if rearrangements involving C chromosomes are taken into account (AN in Table 2). This appears to corroborate the view that during the evolution of divergent avian species from an ancestral form, the original set of group C has remained relatively unchanged, though differences in DNA and its replicational behavior are evident in some allied species (Comings and Mattoccia, 1970; Takagi, 1972). The direction of changes either from low to high or from high to low is still unknown. It might be a sheer coincidence that the corresponding arm number in *Geoclemys reevesii* is 64–68.

Mechanisms of Karyotype Evolution

So far as the 3 A-group chromosomes of birds are concerned, it is now possible to determine the direction of chromosomal changes and to elucidate mechanisms involved. In the following is presented a possible role of centric fission played in karyotype evolution in the *Ciconiiformes* in addition to translocation, pericentric inversion, and centric fusion.

It is probable that karyotypic diversification in the *Ciconiidae* and the *Threskiornithidae* started out with the karyotype similar to the one found in *C. c. boyciana* with the "shared" group A (Fig. 14). The karyotype of this species could give rise to that of *T. melanocephalus* by assuming a centric fission of no. 1 into 2 telocentrics and a reciprocal translocation very close to the centromere between two medium-sized submetacentrics, nos. 6 and 8, into a large submetacentric and a small metacentric. Derivation of the 2 telocentric elements in *T. melanocephalus* from the submetacentric no. 1 is confirmed by their banding patterns. The possibility of a reciprocal translocation close to the centromere between no. 1 and a small biarmed element can not be ruled out in this case, though telocentric nature of the products favors fission mechanism.

It is possible to derive the karyotypes of *N. nippon* and *P. leucorodia* from that of *T. melanocephalus* by a centric fusion between the telocentric element homologous to the short arm of no. 1 and the telocentric no. 11 (Table 5). From a gross morphological basis, a pericentric inversion or a reciprocal translocation is required to explain the difference between the autosomes of *T. aethiopica* and *E. ruber* and the above two species, *N. nippon* and *P. leucorodia*.

Putative examples of centric fission are also found in other orders. It is evident that the pheasant and turkey karyotypes (Stenius *et al.*, 1963) are easily derived from karyotypes of allied species having "shared" group A through a centric fission in the submetacentric no. 2. The karyotype of *Bucephala clangula* (*Anatidae*) consisted of 84 telocentric chromosomes (Hammer, 1970) might be ascribed to centric fissions of nos. 1 and 2 of a karyotype similar to that possessed by *Anas platyrhincus* and *C. chavaria*. Also suggested is that a centric fission in no. 1 might have been one of key steps in the evolution of the owl karyotype from a primitive bird karyotype. Further examples of fission will be found by comparing published karyotypes.

The Robertsonian type of chromosome evolution prevails in a considerable number of taxa both in invertebrates and vertebrates (White, 1973). However, the present consensus of opinion does not necessarily ascribe an important role to centric fission in mammals. It is true that only one possible case of fission has been found (Sinha *et al.*, 1972) thus far, while fusions are aboundant in human populations. Among species other than man, there are sporadic cases which are compatible with a fission hypothesis (Baker *et al.*, 1971; Egozcue, 1971; Fredga and Bergström, 1970; John and Hewitt, 1968; Singh, 1972; Southern, 1969; Todd, 1970; Wahrman *et al.*, 1969; Webster *et al.*, 1972).

Fission implicates that one functional centromere can be split into two functional units. Strong evidence for this event was obtained from

in vitro cloning experiment by Kato *et al.* (1973) in Chinese hamster cell strains. They could isolate a viable clone possessing two telocentrics derived from the X by centric fission. The present findings suggest that the centric fission had played an important role in karyotype evolution in birds. Further accumulation of data will prove frequent participation of the phenomenon in diversification of mammalian karyotypes as well.

Systematic Implications

The present study yielded some important findings relevant to current taxonomic controversies in birds. It is generally believed that the "ratites" consists of at least 5 unrelated groups of birds, which have become flightless secondarily (Mayr and Amadon, 1951; Wetmore, 1960). The concept appears, however, at variance with our present and previous chromosomal findings in the ostrich, rhea, emu and cassowary.

(1) Karyotypes of the 4 species are strikingly similar and apparently interchangeable with one another with slight changes in the centromeric position of one or two macrochromosomal pairs.

(2) No apparently heteromorphic sex chromosome pair is found either in female or in male specimens so far examined.

(3) A segment corresponding to a negative and a positive G-band which proximally locates on the long arm of no. 2 in "carinates" is lacking in the emu and rhea, and possibly in the ostrich and cassowary. This appears responsible for the disproportionately large centromeric index in the ratite no. 2.

(1) is not necessarily a rare event even among remotely related species in birds and cannot be strong evidence. However, (2) suggests that the progenitor of "ratites" had diverged from the main stem of avian evolution before the commencement of morphological specialization of the W chromosome. Furthermore, the possibility of "convergence" is remote in the case of (3), since the karyotype does not seem to respond to selection pressure in the same way as anatomical and physiological characteristics. Our presumptive conclusion is, therefore, the ratite-type no. 2 is derived from a common ancestor of those species. If it had been present in an common ancestor of "ratites" and "carinates" it must have been retained by some present-day "carinates". But, this is not the case so far as our meager data are concerned.

The degree of karyotypic diversification in the family *Accipitridae* and *Falconidae* of the order *Falconiformes* seems extraordinary in view of the general conservatism of avian karyotypes. The typical avian karyotype in the *Cathartidae* of the same order suggests that they have diverged from other falcones for a long time and phylogenetic relationship must be remote as suggested by Ligon (1967).

The present study indicated that the G-banding patterns of the 3 A-group pairs have kept unaltered for the most part over 100 million years. It may be safe to conclude, therefore, that the G-band itself hardly changes in evolution, and it is mostly intra- and/or interchromosomal rearrangements that have been responsible for obscuring chromosomal homology, though the C-band may be subject to frequent change (de Grouchy *et al.*, 1972). Since chromosomal rearrangements are minimal in avian evolution, except for the *Accipitridae* and *Falconidae*, even slight unterspecific chromosomal changes can be detected in a number of cases. The karyotype furnishes phylogenetic evidence independent from anatomical and physiological characteristics. Further extensive cytogenetic analyses together with morphological and biochemical studies are promising to solve certain important systematic problems in birds.

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