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Cytotaxonomy of the Ciconiiformes (Aves), with karyotypes of eight species new to cytology

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Abstract. Somatic karyotypes of 13 species of ciconiiform birds, *Phoenicopterus ruber chilensis, Phoeniconaias minor, Cochlearius cochlearius, Geronticus eremita, Threskiornis molucca, T. spinicollis, Balaeniceps rex, Ciconia ciconia, C. nigra, Euxenura maguari, Xenorhynchus asiaticus, Ephippiorhynchus senegalensis,* and *Leptoptilos crumeniferus* are presented. The chromosomes of eight of these species are described in detail for the first time. Of special interest are a case of structural heterozygosity in a male *B. rex* and remarkably low diploid numbers in *C. nigra* (2n = ca 52) and *L. crumeniferus* (2n = ca 52).

The karyological relationships of the ciconiiform families are discussed. The karyotypes of the Phoenicopteridae are identical to karyotypes found in various other bird orders. All members of the Ardeidae hitherto studied are characterized by a submetacentric third pair of macrochromosomes (subtelocentric in all other Ciconiiformes). All Threskiornithidae share a pair of acrocentric chromosomes resulting from a reciprocal translocation between a pair of microchromosomes and pair No. 1. Both the Ciconiidae and the Balaenicipitidae show the original structure of Nos. 1, 2 and 3, also found in the Phoenicopteridae and many other birds. In contrast to the Phoenicopteridae, however, both families share a relatively high number of medium-sized to small biarmed chromosomes with the Ardeidae and the Threskiornithidae. Several characteristics in this group of chromosomes separate Balaenicipitidae from Ciconiidae.

Introduction

In *Les Chromosomes des Vertébrés* (1949), ROBERT MATTHEY published the first critical review of the cytological literature on all classes of vertebrates, which at the time included data on 133 mammalian and a good two dozen bird species. In two of his last papers MATTHEY (1975, 1976) presented a retrospective of the achievements over forty years in the field of vertebrate cytotaxonomy. The number of mammalian taxa known cytologically had increased to 1458 (approximately 30% of extant species), and the data allowed some general conclusions based on statistical analysis:

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1. Diploid chromosome numbers in eutherian mammals are normally distributed, about 50% of all species having numbers lying between 2n=40 and 2n=56, with a peak at 2n=48;
2. The derivation, from an original modal pool, of karyotypes with diploid numbers lower than the modal ones, can easily be explained by the classical mechanisms of chromosome restructuring; the derivation of karyotypes with higher than modal diploid numbers, however, poses serious problems as it requires de-novo formation of centromeres, large-scale centromeric fissioning being considered unlikely by MATTHEY;
3. There seems to be no correlation between the extent of karyotypical evolution and of morphological evolution; closely related species sometimes have widely divergent karyotypes, and vice-versa (extreme examples illustrating this statement are the well-known cases of *Ellobius, Muntiacus,* etc.);
4. The direction and meaning of karyotype evolution remain enigmatic as long as the problem of the neoformation of centromeres is not solved.

At about the same time WILSON et al. (1974, 1975) and BUSH et al. (1977) estimated the rate of karyotypic evolution in ten mammalian, four reptilian, and two amphibian orders and in teleostean fish on the basis of the number of changes in the numbers of chromosomes and chromosome arms assumed to have taken place in these groups in the course of evolution. These authors found a positive correlation between the rate of karyotypic change and the rate of speciation in these orders. The comparatively rapid evolutionary change in karyotypes of eutherian mammals is ascribed to the small size of demes, a result of the social structure of populations, an important factor being the dependence of the young on the mother for a certain period after birth. The relatively high level of inbreeding due to the small size of demes is thought to have facilitated fixation of chromosomal rearrangements, in accordance with the stasipatric model of speciation of WHITE (1968).

Birds have not been included in these analyses because of the uncertainty in counts of the numbers of their chromosomes and chromosome arms. MATTHEY (1976), discussing bird chromosome numbers, found the sample compiled by RAY-CHAUDHURI (1973) to range from 2n=52 to 2n=98 with a symmetric distribution around a mode of 2n=78, suggesting evolution from an original "pool" of karyotypes with approximately 2n=78. This sample, however, comprised only 86 species. Although many more bird species have been studied cytologically since (DE BOER, in preparation), further data on this vertebrate class with the highest modal diploid number may prove to be of interest. They may also be of interest in view of the problematic origin of chromosome numbers higher than the modal ones, the problem pointed out by MATTHEY.

As Professor MATTHEY has set an example to his students and coworkers by always letting solid facts precede speculation, it seemed fitting to honor the pioneer of vertebrate chromosome research by a contribution to one of the least-developed areas of vertebrate cytotaxonomy.

Cytotaxonomic studies in birds are in a much less advanced stage than those of mammals. Hardly more than 400 of the 9,000 avian species have been studied in some detail cytologically so far, and in most

orders cytological inventories still largely remain undone. Such inventories are of importance in order to obtain rough cytotaxonomic impressions of the various orders and to find out which groups present the most interesting cytological problems and deserve detailed studies involving the application of all the modern banding techniques.

The present communication gives a survey of the chromosome complements of the Ciconiiformes, an order with some 120 recorded species of flamingos, herons, storks, and ibises. Four species of this order have been studied cytologically during the early 1950s by YAMASHINA (1950) and UDAGAWA (1953, 1954), using material from testis sections. Since then, detailed karyotype information based on colchicine-treated material from bone marrow, embryonic tissues, and blood-lymphocyte cultures has been published on 14 species (MORI, 1968; ITOH et al., 1969; HAMMAR, 1970; MISRA, 1974; HOFFMANN et al., 1974; SASAKI and TAKAGI, 1974; TAKAGI and SASAKI, 1974; MISRA and SRIVASTAVA, 1976; OMURA, 1976). The present authors add eight species to this list and reinvestigated the karyotypes of five species that had been studied previously. Apart from the data based on testis sections, at this moment the karyotypes of 22 ciconiiform species are known, including representatives of five of the six families in this order: Phoenicopteridae (flamingos), Ardeidae (herons and their allies), Threskiornithidae (ibises and spoon-bills), Balaenicipitidae (whale-headed stork), and Ciconiidae (storks) (table I). The monotypic family Scopidae (hammer-head) as yet has not been studied cytologically.

From the cytotaxonomic point of view, the Ciconiiformes are an interesting order since, contrary to several other avian groups, they exhibit considerable karyological variability. In addition, the order poses some interesting problems of classification and phylogenetic relationships.

Materials and methods

Chromosome studies were performed of 37 specimens belonging to 13 species: *Phoenicopterus ruber chilensis* (two males), *Phoeniconaias minor* (one male), *Cochlearius cochlearius* (two males), *Geronticus eremita* (two males, two females), *Threskiornis molucca* (two males, two females), *T. spinicollis* (two males, two females), *Balaeniceps rex* (two males, one female), *Ciconia ciconia ciconia* (three males, three females), *C. nigra* (one female), *Euxenura maguari* (two males, two females), *Ephippiorhynchus senegalensis* (one male), *Xenorhynchus asiaticus* (one male), and *Leptoptilos crumeniferus* (three males, one female).

Chromosome preparations were obtained from short-term (three-day) cultures of whole peripheral blood (DE BOER, 1976). Slides were stained with 2% lactic-acetic orcein. Metaphase plates were photographed with a negative magnification of 400×; photographic prints were enlarged to a final magnification of 3000×. A minimal number of 25 cells were analysed for each species, except for *Ciconia nigra, Ephippiorhynchus senegalensis,* and *Xenorhynchus asiaticus* where the quality of the material allowed only about five cells to be analysed.

Cytotaxonomic data

In this review the nomenclature of GRUSON (1976) is followed. The families are treated separately, each including comparisons and discussions of intra-familiar relationships. A general discussion on relationships between the families is given in the next section.

It is difficult to classify bird chromosomes unambiguously according to their

lengths. The classic distinction between "macro"- and "micro"-chromosomes cannot be used in all avian taxa because there is not always a clear-cut boundary between the two. In the karyotypes and their descriptions presented below, the ciconiiform chromosomes are classified in the following groups: large macrochromosomes (first row in the karyotype), medium-sized macrochromosomes (second row), small-to-minute biarmed chromosomes (third row), and acrocentric microchromosomes (including all minute elements of uncertain centromere position). When a pair of small acrocentrics is clearly larger than the next smaller acrocentric microchromosomes, it is placed separately.

Because of the uncertain interspecific homologies of the ciconiiform chromosomes, a uniform numbering system for all species described was not used. The numbers therefore do not necessarily imply homologies between species. Only the numbering system for the first three pairs of large macrochromosomes is based on homologies, since clear evidence from banding studies and comparative chromosome morphology indicates homologies of these pairs between species and families (e.g., TAKAGI and SASAKI, 1974).

Phoenicopteridae

The two species of flamingo hitherto studied cytologically, *Phoenicopterus ruber* (SASAKI and TAKAGI, 1974; TAKAGI and SASAKI, 1974; OMURA, 1976; this report) and *Phoeniconaias minor* (this report), possess karyotypes identical to those found in several other bird orders—all Cathartidae of the Falconiformes, all Gruidae of the Gruiformes, and some Columbidae of the Columbiformes (TAKAGI and SASAKI, 1974; DE BOER, 1976; DE BOER and BELTERMAN, 1980). These karyotypes (fig. 1) are characterized by a diploid chromosome number of approximately 80. The three largest pairs of macrochromosomes have characteristic shapes and are found in representatives of various groups of birds from ratites through passerines. The first pair is metacentric, sometimes showing a weak secondary constriction in the short arm; the second pair is submetacentric, and the third is subtelocentric. Three pairs of medium-sized submetacentrics in the flamingo karyotype include the sex-chromosome pair. The Z chromosome cannot easily be distinguished from the other elements in this group. According to OMURA (1976), the W is a small metacentric element. Except for one pair of small metacentrics (somewhat smaller than the W chromosome), all remaining

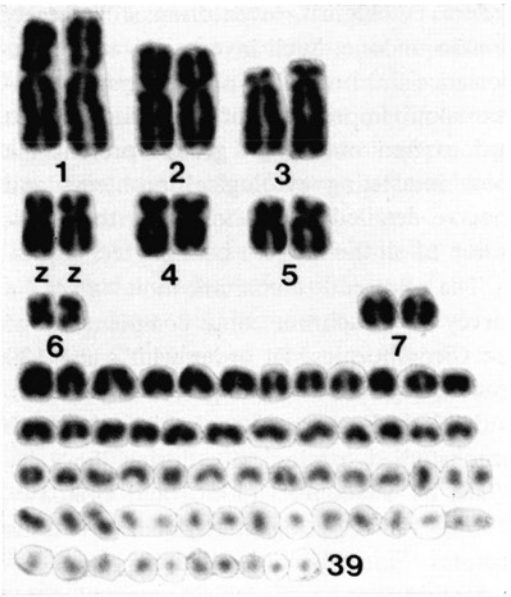


Fig. 1. Karyotype of *Phoenicopterus ruber chilensis* ♂, 2n=80.

Table I. List of the ciconiiform species that have been studied cytologically by using colchicine-treated bone marrow, embryonic cells, or cultured blood lymphocytes. Studies of material from gonadal sections performed during the 1940s and early 1950s are not included. The species are arranged alphabetically in each family.

Family and species	Reference
Phoenicopteridae (flamingos)	
Phoeniconaias minor	this report
Phoenicopterus ruber	TAKAGI and SASAKI, 1974
Phoenicopterus ruber chilensis	OMURA, 1976; this report
Ardeidae (herons and their allies)	
Ardea cinerea	MORI, 1968; ITOH et al., 1969; HAMMAR, 1970; KLEIN, 1973
Ardea purpurea	KLEIN, 1973
Ardeola grayii	RAY-CHAUDHURI, 1967, 1973
Bubulcus ibis	MISRA, 1974; MISRA and SRIVASTAVA, 1976
Cochlearius cochlearius	this report
Threskiornithidae (ibises and spoon-bills)	
Eudocimus ruber	TAKAGI and SASAKI, 1974
Geronticus eremita	this report
Nipponia nippon	SASAKI and TAKAGI, 1974; TAKAGI and SASAKI, 1974
Platalea leucorodia	TAKAGI and SASAKI, 1974 (no figure)
Threskiornis aethiopicus	TAKAGI and SASAKI, 1974 (no figure)
Threskiornis melanocephalus	TAKAGI and SASAKI, 1974 (no figure)
Threskiornis molucca	this report
Threskiornis spinicollis	this report
Scopidae (hammer-head stork)	no cytological data
Balaenicipitidae (whale-headed stork)	
Balaeniceps rex	HOFFMANN et al., 1974; this report
Ciconiidae (storks)	
Ciconia ciconia boyciana	SASAKI and TAKAGI, 1974; TAKAGI and SASAKI, 1974
Ciconia ciconia ciconia	TAKAGI and SASAKI, 1974; this report
Ciconia nigra	this report
Ephippiorhynchus senegalensis	TAKAGI and SASAKI, 1974 (no figure); this report
Euxenura maguari	this report
Leptoptilos crumeniferus	TAKAGI and SASAKI, 1974 (no figure); this report
Xenorhynchus asiaticus	this report

elements are acrocentric (approximately 66) and of gradually decreasing size; the smallest are mere dots. The chromosomes of the largest pairs in this group sometimes show a secondary constriction just below the centromere and tend to form associations in metaphase plates.

Ardeidae (herons and their allies)

The five species of the Ardeidae studied to date (for references see table I) share a characteristic structure of the third pair of macrochromosomes. The first and second pairs are identical to those of flamingos and many other birds. The third pair, however, is subtelocentric in the flamingos but submetacentric in the ardeid species. Since it appears to have the same length in both cases, a pericentric inversion in the ardeid ancestors may have caused this characteristic.

The karyotypes of two species of *Ardea*, *A. cinerea* and *A. purpurea* (RAY-CHAUDHURI, 1967, 1976; MORI, 1968; ITOH et al., 1969; HAMMAR, 1970; KLEIN, 1973), are probably identical, although the diploid chromosome numbers vary somewhat (from 64 to 68). As in the flamingos there are three pairs

of medium-sized chromosomes, including the sex-chromosome pair, though all elements in this group seem to be more metacentric (fig. 2). The W chromosome is a small submetacentric or subtelocentric element. The presence of a pair of medium-sized acrocentrics, somewhat smaller than the chromosomes of the preceding group, is characteristic. There are probably eight pairs of small-to-minute meta- to submetacentric chromosomes. The remaining microchromosomes (approximately 36) are acrocentric and of gradually decreasing size.

The karyotype of *Bubulcus ibis* (MISRA, 1974; MISRA and SRIVASTAVA, 1976) is very similar to those of *Ardea*. The only possible differences are the acrocentric, instead of submetacentric, W chromosome, the somewhat lower diploid number (62), and the presence of fewer minute biarmed elements. As far as the microchromosomes are concerned these differences may be due to technical imperfections. Of special interest is the karyotype of a male *Bubulcus ibis* described by MISRA and SRIVASTAVA, which was heterozygous for a reciprocal translocation between the 1 and a small metacentric chromosome. This translocation (fig. 3) resulted in two new chromosomes: a submetacentric, consisting of the short arm of the original 1, the proximal part of its long arm and a minute part of the small metacentric chromosome, and an almost acrocentric element, consisting of the remainder of the long arm of the 1 and the centromeric region of the small metacentric chromosome.

Ardeola grayii for two reasons forms an exception among the Ardeidae hitherto studied (fig. 4). First, it lacks the normal 2, which appears to be fissioned into two pairs of acrocentric chromosomes corresponding in length to the arms of the 2 of the other species (RAY-CHAUDHURI 1967, 1973, 1976). Second, the W chromosome has almost the same length as the Z, a rather exceptional situation in birds. The former is clearly distinguishable since it is nearly acro-

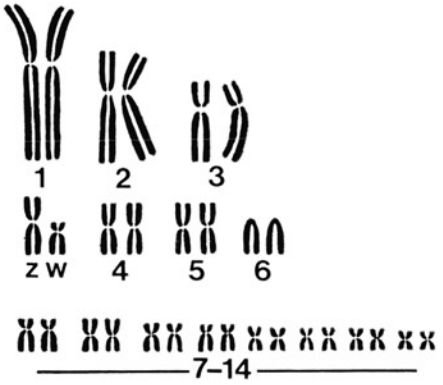


Fig. 2. Macrochromosomes and small biarmed chromosomes of *Ardea cinerea* ♀ (after HAMMAR, 1970).

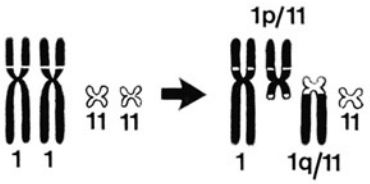


Fig. 3. Reciprocal translocation between chromosome 1 and the small, metacentric 11 found in heterozygous state in a male *Bubulcus ibis* (MISRA and SRIVASTAVA, 1976).

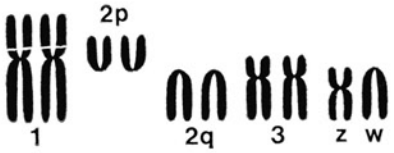


Fig. 4. The first four pairs of macrochromosomes and the sex chromosomes of *Ardeola grayii* (after RAY-CHAUDHURI, 1973).

centric, whereas the Z is (sub)metacentric, as in other ardeids. The absence of one of the pairs of medium-sized submetacentrics and several pairs of small-to-minute biarmed chromosomes may constitute a further difference. However, more detailed studies are necessary to exactly assess the structure of the smaller autosomes.

Together with several other genera *Ardea, Ardeola,* and *Bubulcus* were placed in a separate tribe, Ardeini, of the Ardeinae by BOCK (1956) The chromosome data seem to support putting *Ardea* and *Bubulcus* together, but seem to point to a separate position for *Ardeola.* Therefore the inclusion of *Bubulcus* in *Ardeola,* suggested by BOCK, needs reconsideration.

The taxonomic position of *Cochlearius cochlearius* has been much disputed. It has not been questioned that the monotypic genus *Cochlearius* is ciconiiform, but whether it should be classified together with *Nycticorax* in one tribe of the Ardeidae, in a separate ardeid tribe, in a separate ciconiiform family, or along with *Balaeniceps* and *Scopus,* has been matter of discussion (for a historical review of the classification of Ciconiiformes, see SIBLEY and AHLQUIST, 1972). The submetacentric chromosome 3 of *Cochlearius cochlearius* seems to support the view that we are dealing with an ardeid species; this character has not yet been found in any other family of the Ciconiiformes. For the remainder, however, its karyotype shows little similarity with the ardeid karyotypes. In fact, apart from the morphology of the second pair, it differs only in one respect from the basic karyotype of the flamingos: there are four pairs of medium-sized biarmed chromosomes (fig. 5) instead of three. Since only a single male specimen was studied, the sex chromosomes could not be identified, but the ZZ pair probably is to be found among the medium-sized, biarmed chromosomes. The karyotype of *Cochlearius cochlearius* clearly lacks the pair of medium-sized acrocentrics and the relatively high number of small-to-minute biarmed elements characteristic of *Ardea* and *Bubulcus.* As in the flamingos, there is only one pair of small metacentrics; all the other microchromosomes are acrocentric as far as their centromere position can be identified. The diploid chromosome number is at least 74.

The quality of the chromosome material from gonadal sections of *Egretta garzetta, Nycticorax nycticorax* (YAMASHINA, 1950), *Gorsachius goisagi* (UDAGAWA, 1953), and *Ixobrychus sinensis* (UDAGAWA, 1954) does not allow a comparison with the karyotypes described above. However, the first three

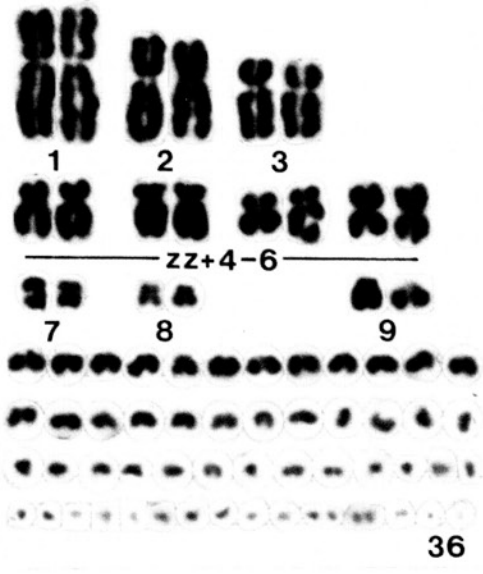


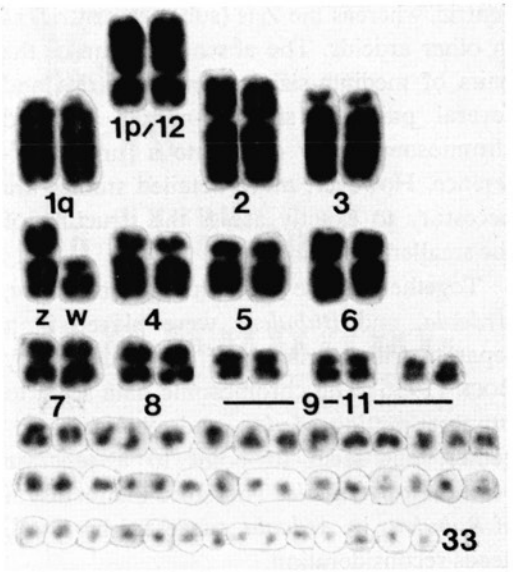
Fig. 5. Karyotype of *Cochlearius cochlearius* ♂, 2n=74.

pairs of macrochromosomes of these species seem to be morphologically identical to those of *Ardea, Bubulcus,* and *Cochlearius.*

Since the few ardeid species studied cytologically constitute a homogeneous group on the basis of their submetacentric 3, but otherwise appear to possess rather heterogeneous karyotypes, further studies on more species would seem promising. Detailed information on karyotype morphology could provide important clues to the obscure relationships within this group.

Threskiornithidae (ibises and spoon-bills)

Whereas the Ardeidae are characterized by the shape of the third chromosome pair, the eight members of the Threskiornithidae (table I) all lack the original metacentric 1 found in all other ciconiiform and many other avian groups. TAKAGI and SASAKI (1974) distinguished three threskiornithid karyotypes, differing in the structure of the remnants of 1 and the presence or absence of a pair of small acrocentrics: **(a)** A karyotype with a pair of acrocentrics corresponding to the long arm of the 1, a pair of acrocentrics corresponding to the short arm of the 1, and a pair of small acrocentrics (designated 1q, 1p, and 12, respectively, in fig. 9; in the numbering system of TAKAGI and SASAKI the small pair is number 10). This karyotype (fig. 9*a*) was found in *Threskiornis melanocephalus* by TAKAGI and SASAKI. **(b)** A karyotype lacking the 12 and showing short arms of the length of 12 attached to the acrocentrics of 1p (fig. 9*b*). This type was found in *Nipponia nippon* and *Platalea leucorodia* by TAKAGI and SASAKI and in *Geronticus eremita, Threskiornis molucca,* and *T. spinicollis* by the present authors (figs. 6, 7, and 8). **(c)** A karyotype identical with the preceding one but with minute short arms attached to the chromosomes of 1q (fig. 9*c*). This type was



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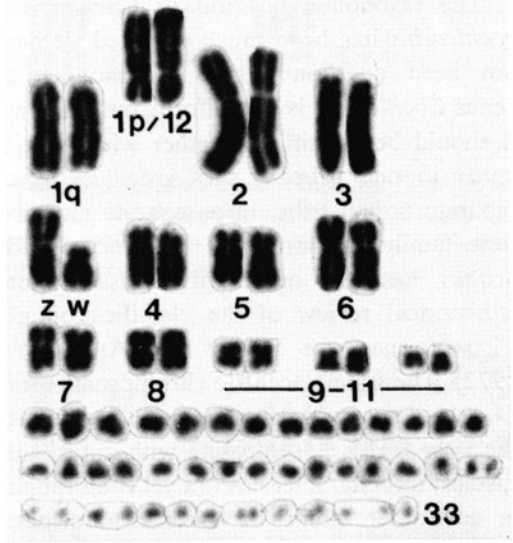

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Fig. 6. Karyotype of *Geronticus eremita* ♀, 2n=68.

Fig. 7. Karyotype of *Threskiornis molucca* ♀, 2n=68.

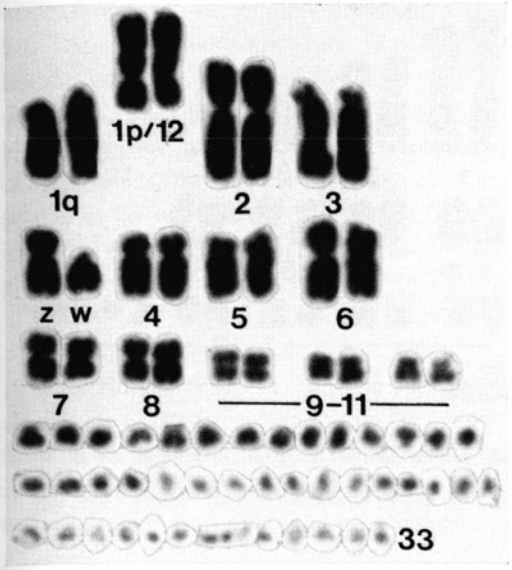


Fig. 8. Karyotype of *Threskiornis spinicollis* ♀, 2n=68.

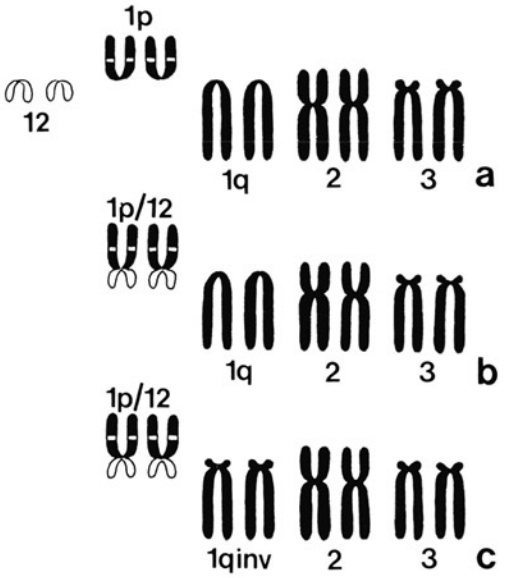


Fig. 9. The homologues of the first three macrochromosomes of the basic karyotype in **a.** *Threskiornis melanocephalus;* **c.** *T. aethiopicus* and *Eudocimus ruber* (after TAKAGI and SASAKI, 1974); **b.** the remaining Threskiornithidae studied cytologically.

found in *Eudocimus ruber* and *Threskiornis aethiopicus* by TAKAGI and SASAKI.

In other respects the karyotypes of the eight species are probably identical. The diploid numbers are approximately 68. The two largest pairs of macrochromosomes are similar to the 2 and 3 of the flamingos. There are four pairs of medium-sized biarmed chromosomes, including the sex chromosomes (the Z is almost metacentric, the W a smaller submeta- to subtelocentric element), two pairs of small metacentrics, and at least three pairs of minute biarmed chromosomes. The largest of the approximately 44 acrocentric microchromosomes measures only half the length of the acrocentric 12 of *Threskiornis melanocephalus*. TAKAGI and SASAKI suggested that the two pairs of large acrocentrics (1p and 1q) of karyotype **a** originated by centric fission of the original 1 or by reciprocal translocation with a microchromosome. Karyotype **b** is supposed to have arisen by fusion between 1p and the acrocentric 12 of *T. melanocephalus*. The homology between pairs 1, 1q, 1p, and 1p/12 was shown by TAKAGI and SASAKI using G-banding. Furthermore, the homology of the short arm of the 1 with 1p is evident by the presence of a weak secondary constriction in both. Karyotype **c** may have arisen by a pericentric inversion in 1q resulting in the minute short arm of this pair.

However, the occurrence of the second type in four species, including two representatives of *Threskiornis,* seems to be an indication that the first type, found only in *T. melanocephalus,* cannot be original. It would seem more logical to posit

that the second type originated by a reciprocal translocation between the original 1 and 12, resulting in the 1q and 1p/12 of fig. 9, and that the karyotype of *T. melanocephalus* evolved secondarily by centric fissioning of the 1p/12, resulting in the 1p and the 12. The minute short arms of 1q in *Eudocimus ruber* and *Threskiornis aethiopicus* must have evolved independently, if the classification of these species in different genera does indeed reflect their phylogenetic relationship. Further studies in the ibises and spoon-bills could throw more light on this matter.

Balaenicipitidae (whale-headed stork)

The sole representative of this family, *Balaeniceps rex,* has a karyotype with an approximate diploid number of 72 (HOFFMANN et al., 1974; this report). The first three pairs of macrochromosomes are of the basic type, identical to those in the flamingos. Four pairs of medium-sized submetacentric to metacentric elements include the sex chromosomes. The Z is submetacentric and cannot easily be distinguished from the other chromosomes in this group; the W is a small metacentric. There are at least six pairs of small-to-minute metacentrics. A pair of small acrocentric chromosomes with satellites (No. 13 in fig. 10) are often found to associate in metaphase plates, with the secondary constriction between the centromeres and the satellites close together. In one of our male specimens the satellites of one of the members of this pair were completely lacking. Nevertheless the elements

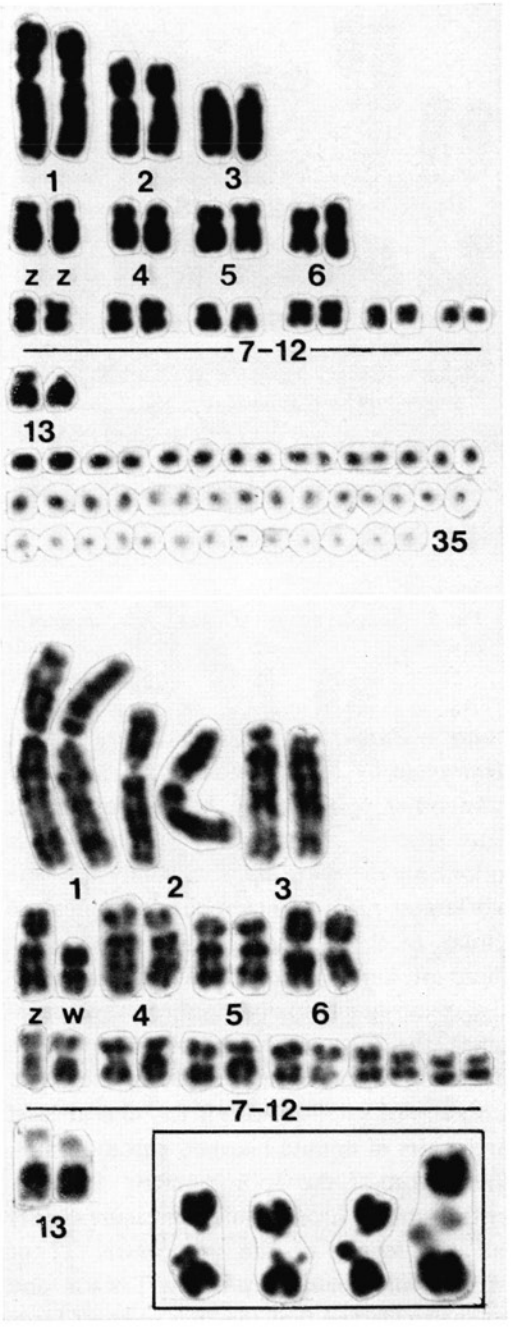


Fig. 10. Karyotypes of *Balaeniceps rex:* top, ♂, 2n=72, heterozygous for a 13 with a satellite; bottom, ♀, homozygous for the 13 with a satellite (modified G banding of pairs 1–13). Detail shows association in the heterozygous male (left three) and in the homozygous female.

were found in association frequently (fig. 10). Another male and one female were homozygous for the chromosomes with the satellites, like the specimens studied by HOFFMANN et al. (1974). The remaining chromosomes are acrocentric microchromosomes as far as their centromeric position is identifiable. The largest elements in this group are less than half as long as the 13.

Ciconiidae (storks)

Like *Balaeniceps rex* and the flamingos, all species of the Ciconiidae show the original first three pairs of macrochromosomes. *Ciconia ciconia* (sspp. *ciconia* and *boyciana*) has a diploid chromosome number of approximately 72 (SASAKI and TAKAGI, 1974; TAKAGI and SASAKI, 1974; this report). The karyotype includes five pairs of medium-sized submeta- to metacentric chromosomes, including the submetacentric Z and the smaller subtelocentric W, one pair of small metacentrics (which in plates with condensed chromosomes cannot always be distinguished from the preceding group), and one pair of minute metacentrics. The remaining chromosomes probably are all acrocentrics; the largest pair (No. 10 in fig. 11) is easily recognizable, as it is clearly longer than the next one. The microchromosomes gradually decrease in size; the members of at least one pair bear tiny satellites and are frequently involved in associations (fig. 11).

The karyotype of *Euxenura maguari,* a species sometimes included in *Ciconia* (e.g., in the checklist of HOWARD and MOORE, 1980), is identical to that of *C. ciconia,* apart from the almost metacentric W chromosome (fig. 12).

Our material of *Ciconia nigra* is not of high quality. Nevertheless, it allows con-

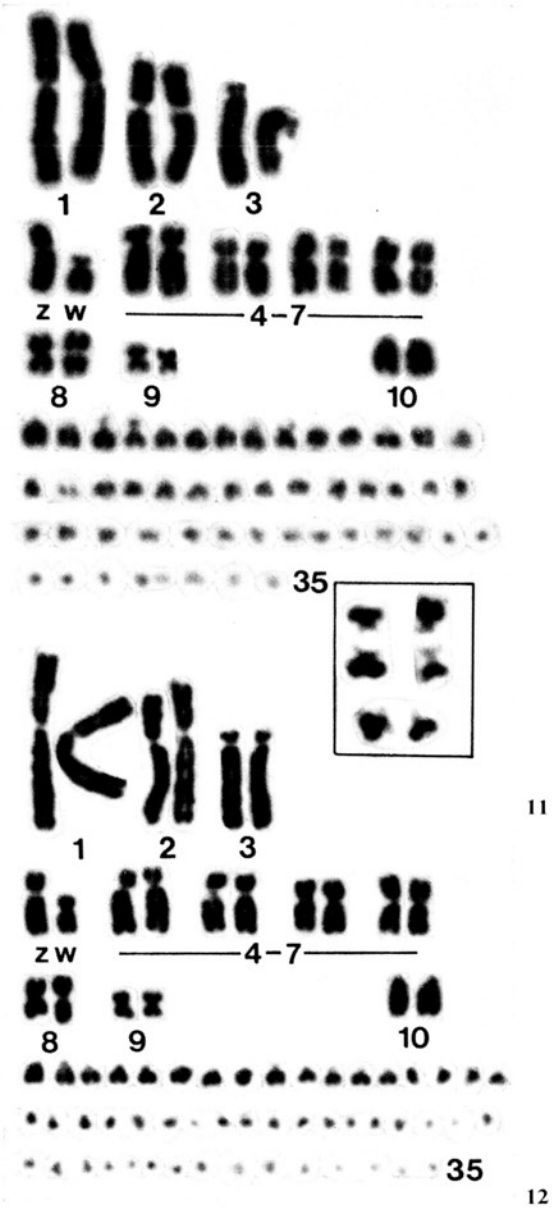


Fig. 11. Karyotype of *Ciconia ciconia ciconia* ♀, 2n=72.

Fig. 12. Karyotype of *Euxenura maguari* ♀, 2n=72.

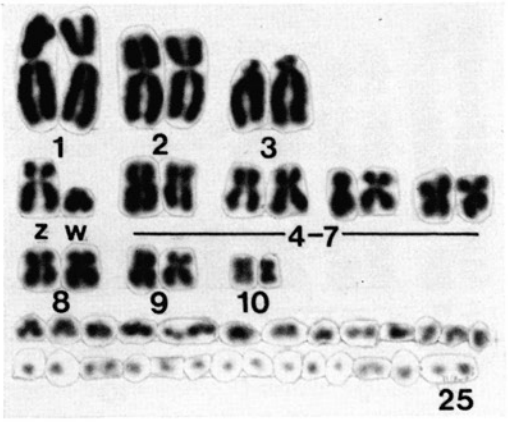
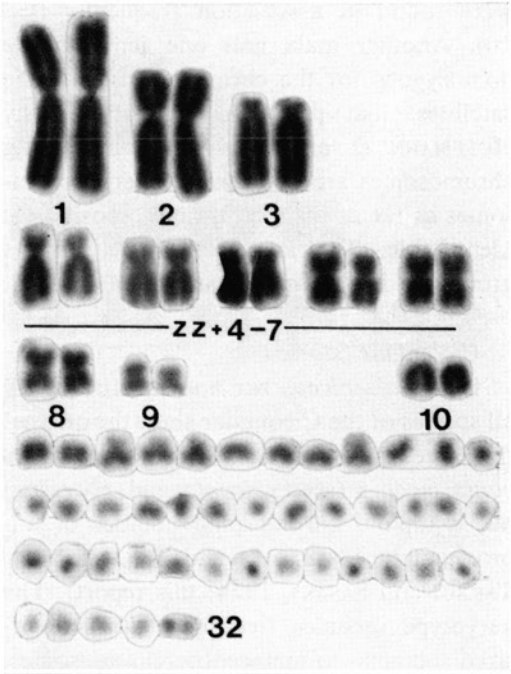


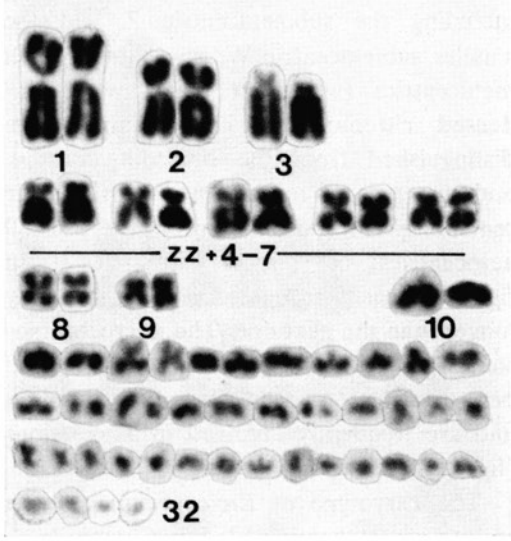
Fig. 13. Karyotype of *Ciconia nigra* ♀, 2n=52.

clusions on the existence of important differences between this karyotype and the two preceding ones. The diploid chromosome number of this species is considerably lower; most probably it does not exceed 2n=52. This difference is almost exclusively due to a large decrease in the number of microchromosomes; the macrochromosomes differ comparatively little from those of *C. ciconia* and *Euxenura maguari*. They include one more pair of medium-sized or small biarmed elements and lack the acrocentric 10 of the previous two species (cf. figs. 11 and 12 with fig. 13). Our present material does not provide any clue to the origin of the great difference in chromosome number.

As far as our relatively poor material allows, we are tempted to conclude that the karyotypes of both *Xenorhynchus asiaticus* (fig. 14) and *Ephippiorhynchus senegalensis* (fig. 15) very much resemble those of *Ciconia ciconia* and *Euxenura maguari*. The macrochromosomes appear to be almost identical; the numbers of microchromosomes could not be established but the diploid number is at least 66 in both species. TAKAGI



14



15

Fig. 14. Karyotypes of *Xenorhynchus asiaticus* ♂, 2n=66.

Fig. 15. Karyotype of *Ephippiorhynchus senegalensis* ♂, 2n=66.

and SASAKI (1974) reported a chromosome number of about 72 for *E. senegalensis*, and a macrochromosome morphology identical to that of *Ciconia ciconia ciconia*. They did not, however, present an illustration of the karyotype.

The diploid chromosome number of *Leptoptilos crumeniferus* was reported to be approximately 70 by TAKAGI and SASAKI, but they did not include a karyotype of this species either. Our studies of four specimens provided over one hundred metaphases of good quality which clearly show that the diploid number definitely does not exceed 2n=52 (fig. 16). The karyotype differs from those of *Ciconia ciconia* and *Euxenura maguari* by the presence of two more pairs of biarmed chromosomes of medium size, and two more pairs of small-to-minute metacentrics, by the absence of the acrocentric 10 of *C. ciconia* and *E. maguari*, and the presence of approximately 12 fewer pairs of acrocentric microchromosomes. The members of one pair of microchromosomes show a secondary constriction just below the centromere and are frequently involved in associations (fig. 16). The Z chromosome is a medium-sized submetacentric; the W, a smaller metacentric. At least in part the low diploid number can be explained by fusions between the original acrocentric 10 and several acrocentric microchromosomes, resulting in four more pairs of biarmed chromosomes. Whether this can explain the disappearance of as many as 24 microchromosomes remains doubtful.

Some authors, like HOWARD and MOORE in their checklist (1980), (based on authors like E. MAYR and J.L. PETERS), recognize only six genera in the Ciconiidae and place them in three tribes: *Ciconia* (including, among others, *C. ciconia, C. maguari* and *C. nigra*) in the Ciconiini; *Ephippiorhynchus* (including *E. senegalensis* and *E. asiaticus*), *Jabiru* and *Leptoptilos* in the Leptoptilini,

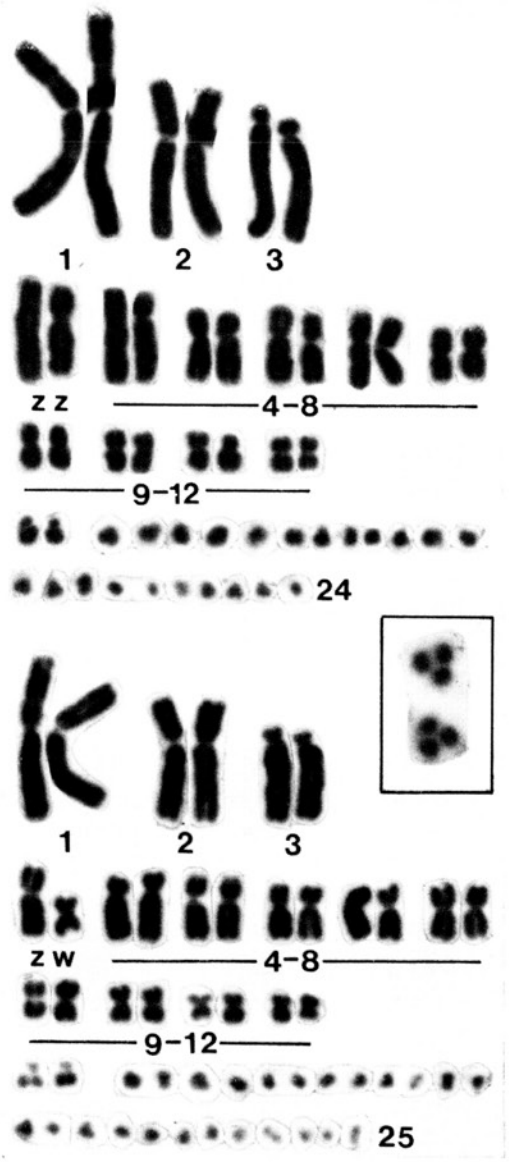


Fig. 16. Karyotypes of *Leptoptilos crumeniferus*, top, ♂, 2n=50, and bottom, ♀, 2n=52. Detail shows association.

and *Mycteria* and *Anastomus* in the Mycterini. The cytological data may make it necessary to reconsider this classification. If the proposed tribal classification were correct this would mean that a considerable reduction in diploid number has taken place independently in the Ciconiini *(C. nigra)* and in the Leptoptilini *(L. crumeniferus)*. Because all other ciconiiform families, and indeed most avian orders, have high chromosome numbers, there seems to be no doubt that the original chromosome number of the Ciconiidae was high. Further detailed investigations of the karyotype of *Ciconia nigra* and other ciconiids are needed to find out whether such a reduction occurred twice or only once.

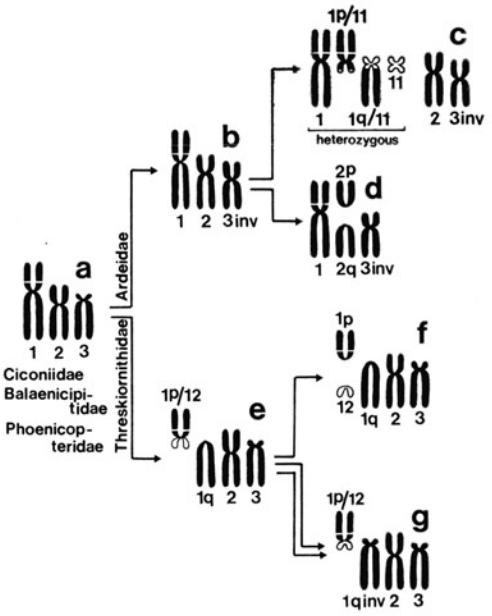


Fig. 17. Possible evolution of chromosomes 1, 2, and 3 in the Ciconiiformes. **a.** original set; **b.** *Ardea* and *Cochlearius*; **c.** *Bubulcus*; **d.** *Ardeola*; **e.** *Geronticus eremita, Nipponia nippon, Platalea leucorodia, Threskiornis molucca,* and *T. spinicollis*; **f.** *T. melanocephalus*; **g.** *T. aethiopicus* and *Eudocimus ruber.*

Discussion

Since karyotypes identical to those of the flamingos are found in various bird orders it is tempting to consider the phoenicopterid karyotype as the original ciconiiform karyotype (assuming that the flamingos naturally belong in this order; see SIBLEY and AHLQUIST, 1972, for a discussion of this question). The idea that this karyotype is original to several avian orders was first expressed by TAKAGI and SASAKI (1974) on the basis of comparisons of banded material.

The Ciconiiformes show an interesting series of transformations in the first three pairs of macrochromosomes: seven different variants of this group exist. Their possible relationships are presented diagrammatically in fig. 17. Both Ardeidae (including *Cochlearius*) and Threskiornithidae (including the spoon-bills, which sometimes have been given separate family rank) constitute clear-cut groups on the basis of transformations of chromosomes in this group: in the former, the 3 deviates from the original, in the latter, the 1. Since these changes are independent, they do not provide information on the relations between the families. It is interesting to note that similar reciprocal translocations occurred independently in the Ardeidae (*Bubulcus ibis* only), and in the Threskiornithidae, involving the original chromosome 1 and a small chromosome. This small chromosome was probably a different one in each case, but the resulting remnants of the 1 are almost identical.

The Balaenicipitidae and Ciconiidae share the original morphology of 1, 2, and 3. The taxonomic position of *Balaeniceps rex* has been much disputed. Most authors agree that this species is ciconiiform, but it has been classified with the Ardeidae, with the Ciconiidae, with *Scopus* and *Cochlearius,* and as a monotypic family, Balaenicipitidae (SIBLEY and AHLQUIST, 1972). COTTAM (1957) was of the opinion that *Balaeniceps rex* should be included in the Pelecaniformes, as a monotypic family. The structure of the first three pairs of macrochromosomes of this species indicates that it does not link up with the Ardeidae, but the occurrence of identical pairs 1, 2, and 3 does not constitute proof of a close relationship between Balaenicipitidae and Ciconiidae, since we are dealing here with an original, plesiomorphic character. It would be of great interest to study the karyotype of *Scopus,* in order to assess its possible relationships with *Balaeniceps* and the other Ciconiiformes.

All Ardeidae, Threskiornithidae, *Balaeniceps,* and the Ciconiidae differ from the flamingos in having higher numbers of biarmed medium-sized and small chromosomes. At this moment it would be premature to speculate on interspecific and inter-family homologies with regard to these elements. Detailed banding studies are necessary to elucidate their origin. Did they evolve partly in common, or independently in the various families? In such an investigation the Pelecanidae (Pelecaniformes) should also be considered, since this group shows some karyological resemblances with the Ciconiiformes (excluding the flamingos). It should be noted, however, that the chromosomes among which clues may be found to possible relationships are very small compared to most mammalian chromosomes, and that therefore only the highest quality banded material can furnish informative data.

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