A karyological study of 55 species of birds, including karyotypes of 39 species new to cytology

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Abstract

The karyotypes of 39 avian species new to cytology are described, viz. *Pelecanus crispus, P. occidentalis* and *Morus bassanus* (Pelecaniformes), *Ardea goliath, Ciconia episcopus* and *Leptoptilos javanicus* (Ciconiiformes), *Anas castanea, Anseranas semipalmata, Cereopsis novaehollandiae, Chloephaga rubidiceps* and *Netta rufina* (Anseriformes), *Falco jugger* and *Milvago chimachima* (Falconiformes), *Aepypodius arfakianus, A. bruijnii, Guttera plumifera, G. edouardi, Lophura edwardsi, L. imperialis* and *Ortalis canicollis* (Galliformes), *Grus rubicunda* (Gruiformes), *Caloenas nicobarica, Goura cristata* and *G. scheepmakeri* (Columbiformes), *Musophaga violacea* (Cuculiformes), *Bubo africanus, Ciccaba woodfordii, Ketupa zeylonensis, Ninox novaeseelandiae, Otus leucotis* and *Phodilus badius* (Strigiformes), *Podargus strigoides* (Caprimulgiformes), *Aceros undulatus, Bucorvus abyssinicus, B. leadbeateri, Buceros bicornis* and *Tockus fasciatus* (Coraciiformes), *Cephalopterus penduliger* and *Picathartes gymnocephalus* (Passeriformes). The karyotypes of 16 additional species are presented for reasons of comparison or due to incomplete descriptions in the previous literature, viz. *Pelecanus onocrotalus* and *Phalacrocorax carbo* (Pelecaniformes), *Ciconia nigra* and *Leptoptilos crumeniferus* (Ciconiiformes), *Anser cygnoides* and *Chauna chavaria* (Anseriformes), *Falco biarmicus* (Falconiformes), *Acryllium vulturinum* (Galliformes), *Grus japonensis, Cariama cristata* and *Psophia crepitans* (Gruiformes), *Bubo bubo, Nyctea scandiaca* and *Tyto alba* (Strigiformes), *Coracias benghalensis* (Coraciiformes) and *Corvus corone* (Passeriformes).

An account is given of the authors' experience with the methodology of culturing avian blood lymphocytes and staining avian chromosomes. The karyotaxonomical implications of the new data are briefly discussed for each individual order.

Introduction

Since 1975 a research project has been conducted in the authors' institution with the two-fold aim of sexing birds for breeding programmes in zoological gardens and nature conservation organizations and obtaining basic data on avian karyology and karyotypic evolution. In the framework of this project the karyotypes of nearly 1000 birds of some 140 different species were studied. To date, these studies resulted in a number of publications describing the karyotypes of individual species or discussing the karyotaxonomy of individual orders (De Boer, 1975, 1976, 1978, 1980; De Boer & Belterman, 1980a, 1980b, 1981; De Boer & Van Brink, 1982; De Boer & Sinoo, 1984; De Boer *et al.*, 1984; Van Dongen & De Boer, 1984).

The present paper brings on record the karyotypes of 55 avian species belonging to 12 different orders, Pelecaniformes, Ciconiiformes, Anseriformes, Falconiformes, Galliformes, Gruiformes, Columbiformes, Cuculiformes, Strigiformes, Caprimulgiformes, Coraciiformes and Passeriformes. Thirty-nine of the species are included because they

Genetica 65, 39–82 (1984).

are new to cytology, a further 9 are included because their karyotypes are poorly documented in the existing literature, and the remaining 7 are included for reasons of comparison. Most of the karyotypes presented here are unbanded. The main reason for this is that all studies were based on chromosome preparations from cultures of peripheral blood lymphocytes, since nearly all birds involved had to be sexed for breeding purposes and therefore had to stay alive. However, the techniques for culturing blood lymphocytes of birds have not yet reached the stage of sophistication they have in mammals: generally the results are rather poor and detailed chromosome banding studies can only be carried out on rare occasions. Nevertheless, it is believed that even the unbanded karyotypes presented here do contribute to our knowledge of the basic karyology of the various orders and families, because the data so far available for most of the larger avian taxa are extremely poor. Such basic karyological knowledge should provide the basis on which more detailed investigations can be undertaken with the aim of solving specific karyotaxonomic problems. Therefore, in the present paper brief discussions on karyotaxonomic relationships are added to the descriptions of the karyotypes of the individual orders and families, pointing to the most interesting problems that might be subject of future studies.

Material and methods

The karyotypes of the 55 avian species presented in this paper are based on the study of 227 birds. The species together with numbers and sex of the animals studied are included in Table 1, which summarizes all avian chromosome studies carried out in the authors' laboratory since 1975. In Table 1 and in the text the nomenclature of the 'Reference list of the birds of the world' (Morony *et al.*, 1975), used by the American Museum of Natural History, is followed.

During the course of the 9 years that avian chromosomes have been studied in our laboratory we have used many variations of the standard procedures for culturing lymphocytes from peripheral blood. Thus, the karyotypes described in this report were obtained with various techniques. So far, no technique has been found to be satisfactory for a wide variety of species: with any of the techniques used great variability of results was observed between and even within species. Below, an indication is given of our experiences acquired from approximately 3 000 peripheral blood cultures of nearly 1 000 birds (some 140 species).

Blood is usually taken from the *vena basilica* at the point where it crossess radius and ulna. Occasionally, particularly in long-legged species, it is obtained from the *vena sphanea* approximately midway the tarso-metatarsus. Approximately 0.1 ml of a sterile solution of Na-heparin (5 000 I.U./ml) is drawn into a disposable syringe prior to taking 0.5 to 2.0 ml of blood. Preferably small syringes are used (up to 2 ml), since the vacuum produced by larger syringes easily causes the *vena basilica* to collapse during drawing. On rare occasions up to 10 ml of blood was drawn from larger birds for special purposes. After drawing, blood and heparin are mixed by gently shaking the syringe. The dust cap is placed on the needle and the blood is left in the syringe until cultures are prepared. The time interval between taking the blood and preparation of the cultures generally varies from 2 h to 2 days. In exceptional cases blood has been cultured with good results 4 days after removal. When cultures are prepared on the same day the blood is kept at room temperature; a temperature of 4 °C is used for longer periods of storage.

Cultures are prepared in 50 ml plastic culture bottles (bottom surface 7 cm^2), placed vertically in the incubator. 0.5 ml of whole blood is used for one culture as a standard. When less blood is available the volume of the culture mixture (see below) is reduced accordingly (good results can on occasions be obtained with as little as 0.2 ml of blood). In some experiments, lymphocytes were separated from red cells either by sedimentation or by the use of Histopaque-1077 (Sigma). In the first case the syringe was placed needle-up for 1 to 3 h, the needle was then carefully bent to form an angle of 120 °C and the serum, containing the white blood cells of 1 ml of blood, was transferred (leaving the syringe upright) to a culture bottle in order to prepare a culture of the usual volume. In the second case, 1 to 2 ml of blood were carefully transferred to a sterile, 10 ml centrifuge tube containing 2 ml of Histopaque-1077 and centrifuged at 1 000 rpm (190 g) for 20 min. The whitish layer in the Histopaque contains the lymphocytes which were then removed

Table 1. List of 141 avian species studied in the authors' laboratory since 1975. Nomenclature is that of Morony *et al.* (1975). When karyotypes were originally described under a synonymic name, this is added in brackets to the reference. For the species whose karyotypes are described in this report the numbers of animals studied are indicated in the form of a code, giving the number of males before the decimal point and the number of females thereafter (e.g. 2.3 = 2 males, 3 females).

Species per order and family		References
STRUTHIONIFORMES		
Struthionidae:	*Struthio camelus* Linnaeus, 1758	unpublished
CASUARIFORMES		
Dromaiidae:	*Dromaius novaehollandiae* (Latham, 1790)	De Boer, 1980
APTERYGIFORMES		
Apterygidae:	*Apteryx australis* Shaw, 1813	De Boer, 1980
PELECANIFORMES		
Pelecanidae:	*Pelecanus crispus* Bruch, 1832	this report (6.5)
	Pelecanus occidentalis Linnaeus, 1766	this report (6.15)
	Pelecanus onocrotalus Linnaeus, 1758	De Boer, 1976; this report (5.5)
Sulidae:	*Morus bassanus* (Linnaeus, 1758)	this report (4.4)
Phalacrocoracidae:	*Phalacrocorax carbo* (Linnaeus, 1758)	this report (1.0)
CICONIIFORMES		
Ardeidae:	*Ardea goliath* Cretzschmar, 1827	this report (1.3)
	Cochlearius cochlearius (Linnaeus, 1766)	De Boer & Van Brink, 1982
Balaenicipitidae:	*Balaeniceps rex* Gould, 1850	De Boer & Van Brink, 1982
Ciconiidae:	*Ciconia ciconia* (Linnaeus, 1758)	De Boer & Van Brink, 1982
	Ciconia episcopus (Boddaert, 1783)	this report (0.1)
	Ciconia maguari (Gmelin, 1789)	De Boer, 1978 *(Euxenura maguari);* De Boer & Van Brink, 1982 *(Euxenura maguari)*
	Ciconia nigra (Linnaeus, 1758)	De Boer & Van Brink, 1982; this report (5.3)
	Ephippiorhynchus asiaticus (Latham, 1790)	De Boer & Van Brink, 1982 *(Xenorhynchus asiaticus)*
	Ephippiorhynchus senegalensis (Shaw, 1800)	De Boer & Van Brink, 1982
	Ibis ibis (Linnaeus, 1766)	unpublished
	Leptoptilos crumeniferus (Lesson, 1831)	De Boer & Van Brink, 1982; this report (4.4)
	Leptoptilos javanicus (Horsfield, 1821)	this report (0.1)
Threskiornithidae:	*Carphibis spinicollis* (Jameson, 1835)	De Boer & Van Brink, 1982 *(Threskiornis spinicollis)*
	Geronticus eremita (Linnaeus, 1758)	De Boer & Van Brink, 1982
	Threskiornis molucca (Cuvier, 1829)	De Boer & Van Brink, 1982
Phoenicopteridae:	*Phoenicopterus chilensis* Molina, 1782	De Boer & Van Brink, 1982 *(P. ruber chilensis)*
	Phoenicopterus roseus Pallas, 1811	unpublished
	Phoenicopterus ruber Linnaeus, 1758	unpublished
	Phoenicopterus minor (Geoffroy, 1798)	De Boer & Van Brink, 1982
ANSERIFORMES		
Anhimidae:	*Chauna chavaria* (Linnaeus, 1766)	this report (6.2)
Anatidae:	*Anas castanea* (Eyton, 1838)	this report (1.0)
	Anser cygnoides (Linnaeus, 1758)	this report (0.2)
	Anseranas semipalmata (Latham, 1798)	this report (1.1)
	Cereopsis novaehollandiae Latham, 1801	this report (0.1)
	Chloephaga rubidiceps Sclater, 1861	this report (1.0)
	Netta rufina (Pallas, 1773)	this report (1.0)
FALCONIFORMES		
Cathartidae:	*Sarcorhamphus papa* (Linnaeus, 1758)	De Boer, 1976
	Vultur gryphus Linnaeus, 1758	De Boer, 1975, 1976
Accipitridae:	*Accipiter gentilis* (Linnaeus, 1758)	De Boer, 1976
	Accipiter novaehollandiae (Gmelin, 1788)	De Boer & Sinoo, 1984
	Aegypius monachus (Linnaeus, 1766)	De Boer & Sinoo, 1984
	Aquila audax (Latham, 1801)	De Boer, 1976
	Aquila rapax (Temminck, 1828)	De Boer & Sinoo, 1984
	Buteo buteo (Linnaeus, 1758)	De Boer, 1976
	Circus aeruginosus (Linnaeus, 1758)	De Boer & Sinoo, 1984

Table 1. continued.

Species per order and family		References
	Circus cyaneus (Linnaeus, 1766)	De Boer & Sinoo, 1984
	Circus pygargus (Linnaeus, 1758)	De Boer & Sinoo, 1984
	Circaetus gallicus (Gmelin, 1788)	De Boer & Sinoo, 1984
	Geranoaetus melanoleucus (Vieillot, 1918)	De Boer & Sinoo, 1984
	Gypaetus barbatus (Linnaeus, 1758)	De Boer, 1976; De Boer & Sinoo, 1984
	Gyps bengalensis (Gmelin, 1788)	De Boer & Sinoo, 1984
	Gyps coprotheres (Forster, 1798)	De Boer, 1975, 1976
	Gyps fulvus (Hablizl, 1783)	De Boer, 1976
	Gyps rueppellii (A. E. Brehm, 1852)	De Boer & Sinoo, 1984
	Haliaeetus albicilla (Linnaeus, 1758)	De Boer, 1976; De Boer & Sinoo, 1984
	Haliaeetus leucocephalus (Linnaeus, 1766)	De Boer & Sinoo, 1984
	Haliaeetus leucogaster (Gmelin, 1788)	De Boer & Sinoo, 1984
	Haliaeetus leucoryphus (Pallas, 1771)	De Boer & Sinoo, 1984
	Haliaeetus vocifer (Daudin, 1800)	De Boer, 1976; De Boer & Sinoo, 1984
	Haliastur indus (Boddaert, 1783)	De Boer, 1976 *(H. indus indus)*
	Lophoaetus occipitalis (Daudin, 1800)	De Boer & Sinoo, 1984
	Necrosyrtes monachus (Temminck, 1823)	De Boer & Sinoo, 1984
	Pernis apivorus (Linnaeus, 1758)	De Boer & Sinoo, 1984
	Pithecophaga jefferyi Ogilvie-Grant, 1896	De Boer, 1976
	Sarcogyps calvus (Scopoli, 1786)	De Boer, 1976
	Stephanoaetus coronatus (Linnaeus, 1766)	De Boer & Sinoo, 1984
	Terathopius ecaudatus (Daudin, 1800)	unpublished
Sagittariidae:	*Sagittarius serpentarius* (Miller, 1779)	De Boer, 1975, 1976
Falconidae:	*Falco biarmicus* Temminck, 1825	De Boer, 1976; this report (2.1)
	Falco jugger Gray, 1834	this report (1.1)
	Milvago chimachima (Vieillot, 1816)	this report (1 unknown sex)
	Polyborus plancus (Miller, 1777)	De Boer, 1975, 1976
GALLIFORMES		
Megapodiidae:	*Aepypodius arfakianus* (Salvadori, 1877)	this report (1.2)
	Aepypodius bruijnii (Oustalet, 1880)	this report (1.1)
Cracidae:	*Crax alector* (Linnaeus, 1766)	unpublished
	Crax mitu (Linnaeus, 1766)	De Boer & Belterman, 1981
	Ortalis canicollis (Wagler, 1830)	this report (1.1)
	Penelope jacquacu Spix, 1825	unpublished
Phasianidae:	*Afropavo congensis* Chapin, 1938	De Boer & Van Bocxstaele, 1981
	Chrysolophus pictus (Linnaeus, 1758)	unpublished
	Gallus domesticus (Linnaeus, 1758)	De Boer *et al.*, 1984 (incl. triploids)
	Lophura edwardsi (Oustalet, 1896)	this report (2.1)
	Lophura imperialis Delacour et Jabouille, 1924	this report (1.0)
	Lophura swainhoii (Gould, 1862)	unpublished
	Pavo cristatus Linnaeus, 1758	unpublished *(P. cristatus* × *Numida meleagris)*
Numididae:	*Acryllium vulturinum* (Hardwicke, 1834)	this report (2.0)
	Guttera plumifera (Cassin, 1857)	this report (2.0)
	Guttera edouardi (Hartlaub, 1867)	this report (9.7)
	Numida meleagris (Linnaeus, 1758)	unpublished *(N. meleagris* × *Pavo cristatus)*
GRUIFORMES		
Gruidae:	*Anthropoides paradisea* (Lichtenstein, 1793)	unpublished
	Anthropoides virgo (Linnaeus, 1758)	De Boer, 1975, 1976
	Balearica pavonina (Linnaeus, 1758)	unpublished *(B. p. pavonina* and *B. p. regulorum)*
	Bugeranus carunculatus (Gmelin, 1789)	unpublished
	Grus antigone (Linnaeus, 1758)	unpublished
	Grus canadensis (Linnaeus, 1758)	unpublished
	Grus grus (Linnaeus, 1758)	unpublished
	Grus japonensis (P. L. S. Müller, 1776)	this report (3.5)
	Grus ribicunda (Perry, 1810)	this report (1.1)
	Grus vipio Pallas, 1811	unpublished

Table 1. continued.

Species per order and family		References
Psophiidae:	*Psophia crepitans* Linnaeus, 1758	this report (2.0)
Rallidae:	*Rallus philippensis* Linnaeus, 1766	unpublished
Cariamidae:	*Cariama cristata* (Linnaeus, 1766)	this report (2.3)
	Chunga burmeisteri (Hartlaub, 1860)	unpublished
COLUMBIFORMES		
Columbidae:	*Caloenas nicobarica* (Linnaeus, 1758)	this report (4.4)
	Goura cristata (Pallas, 1764)	this report (12.11)
	Goura scheepmakeri Finsch, 1875	this report (3.3)
	Goura victoria (Fraser, 1844)	De Boer & Belterman, 1980b
PSITTACIFORMES		
Cacatuidae:	*Cacatua galerita* (Latham, 1790)	Van Dongen & De Boer, 1984
	Calyptorhynchus magnificus (Shaw, 1790)	Van Dongen & De Boer, 1984
	Probosciger aterrimus (Gmelin, 1788)	Van Dongen & De Boer, 1984
Psittacidae:	*Amazona ochrocephala* (Gmelin, 1788)	De Boer & Belterman, 1980a
	Amazona viridigenalis (Cassin, 1853)	Van Dongen & De Boer, 1984
	Ara ararauna (Linnaeus, 1758)	Van Dongen & De Boer, 1984
	Ara macao (Linnaeus, 1758)	Van Dongen & De Boer, 1984
	Melopsittacus undulatus (Shaw, 1805)	Van Dongen & De Boer, 1984
	Nestor notabilis Gould, 1856	De Boer & Belterman, 1980a
	Psittacus erithacus Linnaeus, 1758	De Boer & Belterman, 1980a
	Psittrichas fulgidens (Lesson, 1830)	Van Dongen & De Boer, 1984
CUCULIFORMES		
Musophagidae:	*Musophaga violacea* Isert, 1789	this report (0.1)
STRIGIFORMES		
Tytonidae:	*Phodilus badius* (Horsfield, 1821)	this report (1.0)
	Tyto alba (Scopoli, 1769)	this report (2.2)
Strigidae:	*Bubo africanus* (Temminck, 1821)	this report (0.1)
	Bubo bubo (Linnaeus, 1758)	De Boer, 1976 *(B. bubo bubo);* this report (0.1)
	Ciccaba woodfordii (A. Smith, 1834)	this report (0.1)
	Ketupa zeylonensis (Gmelin, 1788)	this report (1.0)
	Ninox novaeseelandiae (Gmelin, 1788)	this report (1.1)
	Nyctea scandiaca (Linnaeus, 1758)	this report (1.1)
	Otus leucotis (Temminck, 1820)	this report (0.1)
CAPRIMULGIFORMES		
Podargidae:	*Podargus strigoides* (Latham, 1801)	this report (0.1 + 2 unknown sex)
CORACIIFORMES		
Alcedinidae:	*Dacelo novaeguineae* (Hermann, 1783)	De Boer & Belterman 1980b *(Dacelo gigas)*
Coraciidae:	*Coracias benghalensis* (Linnaeus, 1758)	this report (2.4)
Bucerotidae:	*Aceros undulatus* (Shaw, 1811)	this report (1.5)
	Buceros bicornis Linnaeus, 1758	this report (0.1)
	Bucorvus abyssinicus (Boddaert, 1783)	this report (1.1)
	Bucorvus leadbeateri Vigors, 1825	this report (0.1)
	Tockus fasciatus (Shaw, 1811)	this report (1.0)
PASSERIFORMES		
Cotingidae:	*Cephalopterus penduliger* Sclater, 1859	this report (6.4)
Muscicapidae:	*Picathartes gymnocephalus* (Temminck, 1825)	this report (1 unknown sex)
Fringillidae:	*Serinus canarius* (Linnaeus, 1758)	unpublished
Ploceidae:	*Passer domesticus* (Linnaeus, 1758)	unpublished
Corvidae:	*Corvus corax* Linnaeus, 1758	unpublished
	Corvus corone Linnaeus, 1758	this report (0.1)

using a sterile Pasteur's pipette, washed once in 3 ml of culture medium and spun down at 900 rpm. The cells were used to prepare a culture of the usual volume.

The culture mixture is composed of the following components:

(a) 5.0 ml of culture medium. Several media have been used: TC 199, Ham F10, Ham F12 and RPMI 1640 (either Gibco, Difco or both makings). Constant, significant differences in results were not noted. Recently, however, Iscove's Medium (a serum containing medium of Gibco) was tested and found to be superior to all other media previously used. It has since been used in some 200 cultures of 100 birds (20 species) and the average results have improved significantly.

(b) 1.0 ml of serum. Usually desiccated calf serum (Difco) is used. Other sera (chicken serum, various self-made other avian sera) were tested but did not yield significantly different results. Also the quantity of serum added was varied without revealing conclusive evidence as to the optimal concentration. Since the currently preferred Iscove's medium contains 20% serum, no extra serum is added.

(c) 0.1 to 0.2 ml of mitogen. Several mitogens have been tested: phytohaemagglutinin (Difco, Gibco, Wellcome or self-made), pokeweed mitogen (Gibco), Concanavalin-A (Sigma or self-made) and Lypopolysaccharide (Difco). The mitogenic effects of the last three products, with a few exceptions, proved to be low, often even nil. Of these three, pokeweed mitogen in a dose of 0.15 ml (dissolved according to instructions on Gibco label) appeared to give the best chance of yielding at least some mitoses in a wide variety of species. The mitogenic stimulation can often be slightly improved when a second dose of pokeweed mitogen is added after 24 h of incubation. Nevertheless, results are rather unpredictable and vary from species to species and from specimen to specimen. Best mitogenic stimulation in birds of many different taxa is undoubtedly obtained by using phytohaemagglutinin (generally applied in a dosage of 0.1 ml, after dissolving according to instruction on label). However, in many birds this mitogen also is responsible for excessive agglutination of blood cells, causing one big clot of cells to be formed in the culture. By no means can reasonable numbers of undamaged cells be obtained from such clots. The occurrence of the effect of excessive agglutination varies from species to species, and for every species in which larger numbers of animals were studied there appeared to be great individual differences between specimens. An attempt was made to reduce the risk of excessive agglutination by lowering the dosage of phytohaemagglutinin stepwize to 0.01 ml per culture. However, the conclusion of these experiments was, that the mitogenic effect is reduced as quickly as the agglutinating effect when less phytohaemagglutinin is used. In order to prevent the ill effects of excessive agglutination, cultures were prepared which contained no erythrocytes. This was done either by using serum containing white blood cells from sedimentated blood, or leucocytes separated with the aid of Histopaque (see above). Although very good results were obtained on occasions, it appeared that even in these cultures excessive agglutination may be caused by phytohaemagglutinin. Therefore, since the separation of white blood cells in the limited volumes of blood often available (less than 1.0 ml) is hardly practicable, whole blood cultures were preferred. Recently, it has been discovered that the agglutinating effect of phytohaemagglutinin is considerably reduced when whole avian blood is cultured in Iscove's medium. In this medium excessive agglutination is only found in approximately 20% of the cultures (still with variation from species to species and from specimen to specimen). Currently, whenever possible at least two cultures were prepared for each specimen, the first with Iscove's medium and phytohaemagglutinin (0.1 ml), the second with the same medium and a repeated dose of pokeweed mitogen (0.15 ml at the beginning of incubation and 0.1 ml 24 h later). Whenever excessive agglutination is found in the first culture, the second culture acts as a 'second security' since, although mitotic frequencies obtained with pokeweed mitogen are generally low, agglutination is never found in such cultures.

(d) 0.1 ml of a penicillin/streptomycin solution (Gibco), containing 10 000 U/ml of penicillin and 10 000 mcg/ml of streptomycin. This is only added in cases of suspected contamination.

(e) 0.1 ml of a 10% solution of $NaHCO_3$ from time to time has been added to larger series of cultures. No significant improvement of results was found. The same is true for the application of 5% CO_2 gas in the culture bottles. The pH of the cultures with Iscove's medium, phytohaemagglutinin or pokeweed mitogen and penicillin/streptomycin is 7.4 before the blood is added.

Incubation temperatures were varied from 37 to

41 °C. Good results were obtained with all of these temperatures, but on the average 41 °C seemed to be slightly better than lower temperatures. Incubation time was generally approximately 72 h. Sister-chromatid exchange experiments in several species showed that, even in cultures with low mitotic frequencies, a relatively high proportion of the stimulated cells are in the 5th or 6th mitotic cycle after three days of incubation, indicating that avian cell cycle under the given circumstances is shorter than that of mammals. This also indicates that the incubation time should not necessarily be a multiple of 24 h.

One hour before termination of incubation 0.1 ml of a 0.005% solution of colchicin (BDH) is added. Longer incubation times with colchicin were found to result in extreme condensation of all metaphase plates. Even the 1 h colchicine treatment easily causes a 'colchicine-effect' in the small microchromosomes resulting in the separation of their chromatids. Even after 1 h of colchicine treatment most of the metaphase plates often are too much condensed, particularly for chromosome banding studies. Therefore, experiments were carried out to obtain prometaphase chromosomes by adding 0.05 ml of a solution of 0.005 mg/ml methotrexate (Lederle) in medium 20 h before termination of the cultures. Seven h before termination the cells were spun down (1000 rpm, 5 min) and fresh culture medium was added. One h before termination, as usual, colchicine was added. The addition of methotrexate, however, reduced the mitotic frequency (which under normal conditions generally already is rather low) to almost zero.

Cultures are fixed according to the following procedures:

(a) Cells are resuspended in the culture mixture and transferred to a 10 ml centrifuge tube. If the culture contains persistent clots no special attempts are made to disperge them. Resuspending is performed by gently stirring with a Pasteur's pipette.

(b) Cells are spun down for 5 min at 1000 rpm (190 g); the supernatant is discarded, a residue of approximately 1 ml is left.

(c) Cells are resuspended in 5 ml of 0.075 M KCl and placed in a waterbath of 41 °C for 10 min; the suspension is stirred from time to time with a Pasteur's pipette.

(d) Cells are spun down (5 min, 1000 rpm), the supernatant is discarded until 1 ml of residue is left. Cells are resuspended in the residue, and while continuously mixing with a Pasteur's pipette a few drops of Carnoy's fixative (3 : 1 methanol : acetic acid 99%, freshly prepared) are added. After 10 s, somewhat more rapidly, the total volume of fixative is brought to 5 ml. It is our experience that the addition of the first drops of fixative in birds should be done much more carefully than in mammals, the risk of coagulation being much greater.

(e) The fixed cells are spun down (5 min, 1000 rpm) and the supernatant is discarded. The residue is much larger than in mammalian cultures, since the red blood cells do not disappear during KCl hypotonic treatment. With bird cultures it regularly happens that the tube for the larger part is filled with a gel-like structure. In such cases half of the contents of the tube is discarded.

(f) Cells of the residue are resuspended in 5 ml of fixative and spun down again. If a gel was present after the first fixation, this mostly disappears after second fixation and a clear demarcation between supernatant and sediment is seen. Generally fixation has to be repeated a third time, sometimes even a fourth before a relatively clear supernatant is obtained. It will, however, never be as clear as that in material from mammalian cultures.

(g) After the last centrifugation the volume of packed cells varies between 0.5 and 1.0 ml. Depending on this volume the cells are resuspended in 2 to 4 ml of freshly mixed fixative. Slides are made either by flame drying (using cold wet slides) or by air drying (using cleaned dry slides). Cell suspensions in Carnoy's fixative may be stored at -20 °C for over one year. In such cases good spreads are still obtained when cells are spun down and fresh fixative is added just prior to preparing the slides.

The chromosome preparations are generally stained with lacto-aceto orcein (15 min in a 2% solution of orcein in a mixture of 1:1 70% lactic acid and 45% acetic acid, followed by dehydration in a progressive ethanol series and inclusion in Malinol via a xylene bath) or with Giemsa (5 min in 4% of Giemsa stock solution in Sörensen buffer, pH 6.9, followed by air drying and inclusion in Malinol via a xylene bath).

Slides prepared from avian blood cultures in the above manner contain large numbers of erythrocytes relative to the small number of white blood cells and the even much smaller number of mitoses. This means that the metaphases are not easily de-

tected, the more so since avian metaphase plates are much smaller than mammalian ones (on the average the avian nuclear DNA content is only some 40% of that of mammals; Atkins *et al.*, 1964). Due to the low numbers of mitoses per slide, screening should be undertaken using the lowest possible magnification (objective 10×, oculars 8–10×). This, however, requires great experience because of the small size of the chromosomes and the enormous numbers of erythrocytes. Nevertheless, once such experience is gained good metaphases are often quickly found even in very poor cultures.

The small size of avian chromosomes also causes great problems in chromosome banding studies. While avian nuclear DNA content is only 40% of that of mammals, the average bird karyotype has twice the number of chromosomes found in the average mammalian karyotype. Thus, on the average bird chromosomes are only one fifth the length of mammalian chromosomes. In most avian karyotypes only two or three pairs are larger than the mammalian X, while often fewer than 8 pairs are larger than the smallest human chromosome 22. This means that banding techniques yielding acceptable results in mammalian material are generally rather disappointing in birds, resulting in the individual recognition of only a few of the largest pairs. In normal metaphases all other chromosomes are too small to yield characteristic banding sequences. Reasonable results are only obtained in the highest quality metaphase spreads with long chromosomes. It is only in recent years that in the domestic chicken – the subject of the vast majority of studies on avian chromosomes – with the use of the most sophisticated techniques some 15 of the 37 chromosome pairs can be individualized by their banding pattern (see for instance Sasaki, 1981). In other species, the results of chromosome banding on material of routine blood cultures are much poorer. Below, a brief account is given of our experiences with the usual banding techniques in birds.

C-banding (Fig. 1-c) is performed by treatment of the slides in a saturated solution of $Ba(OH)_2$ in destilled water at 60 °C, followed by rinsing in cold distilled water, treatment in 2 × SSC at 60 °C for 20 min, rinsing in distilled water and staining in 10% Giemsa stock solution in Sörensen buffer (pH 6.9). The optimal duration of the $Ba(OH)_2$ treatment was found to be rather variable with bird chromosomes (3 to 10 min). In contrast to most other banding techniques C-banding in birds works best on moderately condensed chromosomes. The amount of C-positive material in birds is very small: the large chromosomes generally show only minute segments of centromeric heterochromatin, some of which are not even consistently seen in all metaphases, while only a part of the microchromosomes is C-positive. In many species the W-chromosome is the only element that is consistently very clearly stained after C-banding. C-banding is preferably done on slides that are at least three days old.

Q-banding is performed by 15 min staining in a solution of approximately 0.05% quinacrine-dihydrochloride in destilled water, followed by rinsing under running tap water and mounting in destilled water. Q-banding requires very long (pro)metaphase chromosomes, otherwise only a few bands are seen in the largest two or three pairs. Contrast between bright and faint bands, even in long chromosomes, is relatively low.

G-banding (Fig. 1-d) is preceded by 15 s treatment of the slides in 3% hydrogen peroxide and rinsing in distilled water. The slides are then treated with a 0.25% solution of Trypsin 1:250 (Difco) in Ca-Mg-free Hank's solution (Difco) (pH 7.2) for approximately 3 s, followed by rinsing in distilled water and staining in 4% Giemsa in Sörensen buffer for 5 min. As with Q-banding, G-banding in birds requires very long chromosomes.

Sister-chromatid exchange studies (Fig. 1-e/f) are performed by adding 0.2 ml of a 0.025% solution of BUdR (Sigma) in culture medium to the culture mixture at beginning of incubation. Chromosome preparations are stained for 15 min in a solution of 0.005 g of Bisbenzimid H33258 (Riedel de Haën) in 100 ml of phosphate buffered saline (pH 7.0). Slides are rinsed in buffered saline and placed in a dish with the same buffer under a TUV 80 W lamp (Philips) for 60 min (distance from lamp to slide 50 cm). They are rinsed in distilled water and treated with 2 × SSC at 65 °C during 90 min, rinsed in distilled water and stained in 10% Giemsa in Sörensen buffer (pH 6.9) during 10 min, rinsed in destilled water, dried and mounted in Malinol via a xylene bath. As after three days of culturing avian lymphocytes often appear to have passed four or more cell cycles (Fig. 1-f), sister chromatid exchange studies can be better undertaken after 2 days of incubation.

BUdR-R-banding (Fig. 1-b) is performed by ad-

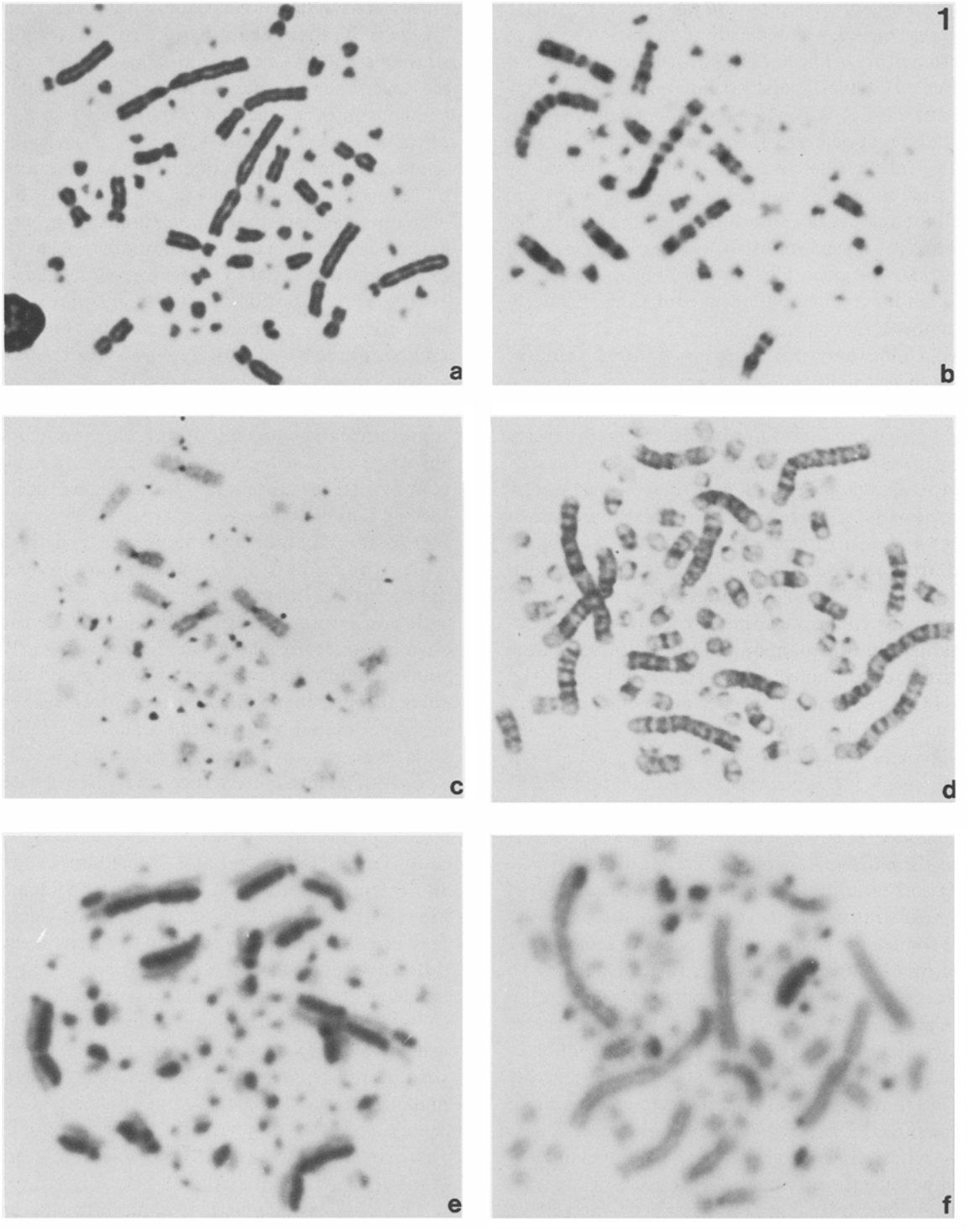


Fig. 1. Examples of the results of various staining techniques in avian chromosomes: (a) orcein stain *(Leptoptilos javanicus);* (b) BUdR-R-banding *(Gallus domesticus);* (c) C-banding *(Balearica pavonina);* (d) G-banding *(Aepypodius arfakianus);* (e) sister-chromatid-exchange (*Grus vipio*, second metaphase); (f) sister-chromatid-exchange (*Grus vipio*, fourth or fifth metaphase from 72-h culture).

ding 0.5 ml of a 0.1% solution of BUdR (Sigma) in culture medium 4–5 h before termination of avian blood cultures. The staining procedure is the same as that for sister-chromatid exchange studies.

So far, NOR-banding on bird chromosomes was only rarely done in our laboratory, and results were poor. A 50% silver nitrate solution is dropped on the slide which is covered with a cover slip and incubated in a moist chamber during 12 h at 65 °C. The slide is rinsed in distilled water and air dried. Contrasts are generally low and silver grains not always distinguishable from dot-shaped microchromosomes.

Microphotographic techniques used for the study of avian chromosomes are similar to those in use for mammals. Photographs are taken on Agfa copex pan rapid film (fluorescent stainings) or Agfa copex pan film (all other stainings), using a Zeiss III photomicroscope with III RS fluorescence equipment. Planapo objectives are used for orcein or Giemsa stained preparations, Neofluar objectives for fluorescent stainings. Magnification on the negatives is 400×, prints are enlarged to 3 000×. The smallest microchromosomes are most easily detected when lower gradations of photographic paper are used.

Results and discussion

The karyotypes of the 55 avian species included in this study are described per order below. Discussions on possible karyological relationships within or between families are included in the treatment of each individual order. No general discussion is added since the material presented here hardly allows conclusions on interorder relationships.

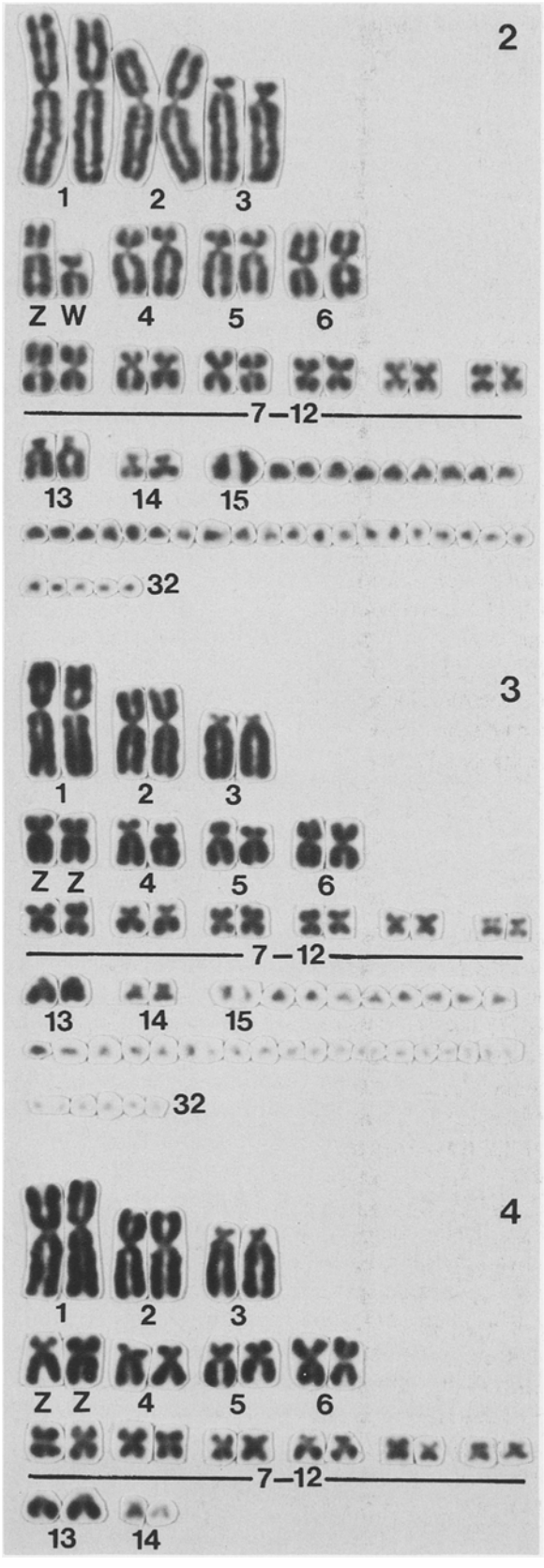
Generally, no attempts have been made to number the chromosome pairs of individual species according to a uniform system. In the absence of extensive detailed banding studies this is considered to be unjustified. Numbering systems based on presumed interspecific homologies are used only in closely related species and in some cases of clear morphological similarity.

In the illustrated karyotypes all the detectable chromosomes of the corresponding metaphase plates are shown and not only the larger chromosomes. However, the number of chromosomes shown in an illustration does not necessarily exactly correspond with the diploid number for the species given in the text. The diploid number counted in avian metaphases depends greatly on the quality of the plates and the degree of condensation of the chromosomes (because of the extremely small size of the shortest elements). Therefore, generally we tend to attribute more value to the highest number of chromosomes counted in good quality plates with both long chromosomes and no apparent colchicine effect (causing separation of the chromatids of the small elements so that many of them erroneously may be counted as two chromosomes), rather than giving the modal diploid number of a larger number of metaphases of a lower average quality.

Since in many cases there is no clear distinction between macrochromosomes and microchromosomes the arrangement of the chromosomes in these two major categories, often practiced in birds, is not strictly followed here. In the karyotypes described the chromosomes are arranged in groups of pairs that can be clearly distinguished from those of other groups. This means that the grouping may vary from species to species, although generally the following groups are distinguished: (1) large macrochromosomes (roughly representing over 7% of the total haploid Z and microchromosomes containing chromosome length); (2) medium-sized macrochromosomes (roughly between 3% and 7% TCL); (3) small biarmed chromosomes (less than 3% TCL); and (4) small to minute acrocentric (micro)chromosomes including all elements of undetectable centromeric position. Whenever appropriate, arm-ratios (length of the long arm divided by that of the short arm) of clearly recognizable chromosomes are given in the text. Chromosomes with arm ratios between 1.0 and 1.6 are referred to as metacentric, those with arm ratios between 1.6 and 3.0 as submetacentric, those with arm ratios exceeding 3.0 as subtelocentric when a distinct short arm is seen or otherwise as acrocentric. Unless indicated otherwise, all illustrated karyotypes are shown in a final magnification of 3 000×.

Pelecaniformes

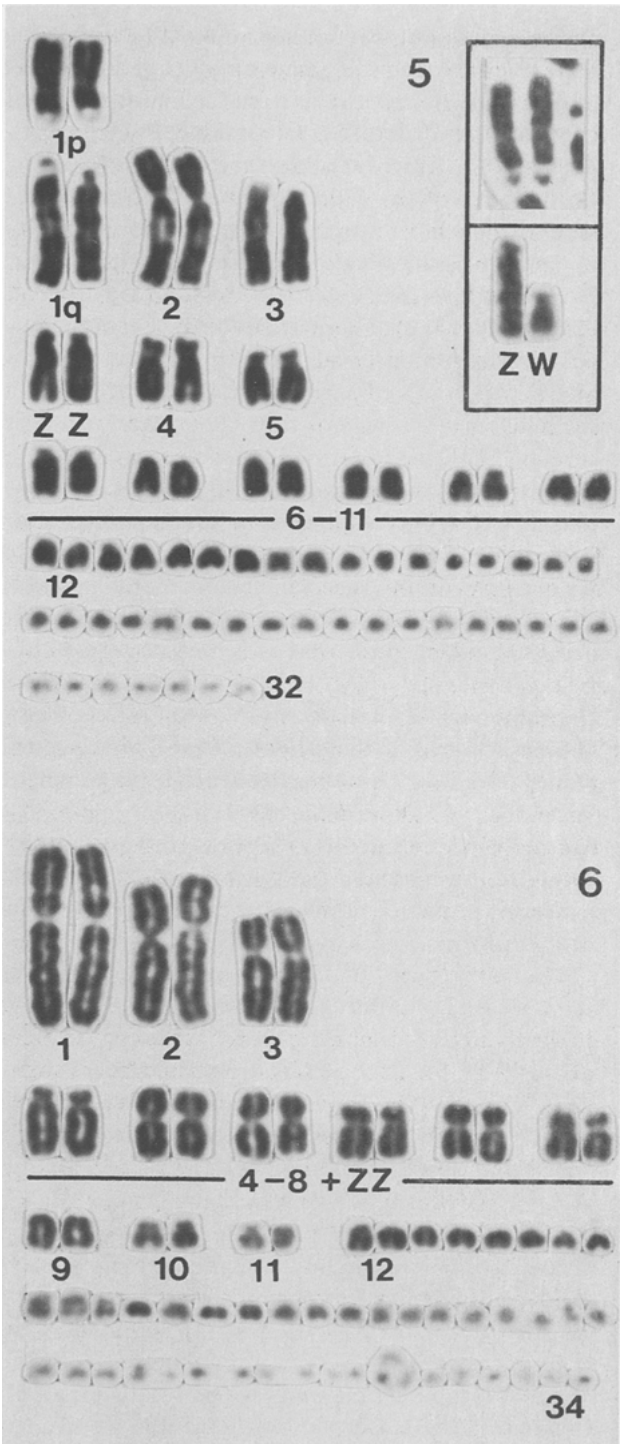
The karyotypes of the two hitherto unstudied pelecans, *Pelecanus crispus* and *P. occidentalis* (Figs. 2 and 3) (Pelecanidae), are identical to that of *P. onocrotalus* (Fig. 4) described previously (Takagi & Sasaki, 1974; De Boer, 1976). Their diploid



chromosome numbers are 66 or 68. The first three pairs of large macrochromosomes are of a characteristic morphology that is found in many species of various other orders (e.g. Ciconiidae, Balaenicipitidae and Phoenicopteridae of the Ciconiiformes, Cathartidae of the Falconiformes, Gruidae of the Gruiformes, most Anseriformes, and some species of Columbiformes; Takagi & Sasaki, 1974; De Boer, 1976; De Boer & Belterman, 1980b; De Boer & Van Brink, 1982). There are two pairs of submetacentric medium-sized macrochromosomes (pairs 4 and 5), while a third pair of similar size (no. 6) has a median centromeric position. There are six pairs (nos 7–12) of small biarmed autosomes all of which, except pair 8, are metacentric. Pair 13 is acrocentric, and clearly recognizable. The remaining autosomes are of small to minute size. All of them are acrocentric or of uncertain centromeric position except pair 14, which shows small but distinct short arms. The Z-chromosome is a medium-sized submetacentric, and is not always distinguishable from the members of pairs 4 and 5. The W is a small submetacentric of the size and shape of the autosomes of pair 8. These results are in full agreement with those of Takagi & Sasaki (1974) for *P. onocrotalus*. Theodorescu (1975) reported fewer small biarmed chromosomes for the same species. This, however, most probably is due to the quality of his preparations.

The karyotype of *Morus bassanus*, the first member of the family Sulidae to be studied cytologically, differs considerably from that of the pelecanids (Fig. 5), although its diploid number is similar ($2n = 68$). The large metacentrics of pair 1 are absent; instead, two pairs of acrocentrics are found with banding patterns similar to those of the long and the short arm of pair 1 in the pelicans (Fig. 7). Thus, because of the probable homology, these acrocentrics are designated 1q and 1p respectively. As in the short arm of chromosome 1 of the pelicans, in 1p of *Morus bassanus* a secondary constriction is sometimes seen approximately 1/3 the distance from centromere to telomere (Fig. 5). C-banding, however, shows that the combined amount of centromeric heterochromatin of 1p and

Figs. 2–4. Representative karyogrammes of Pelecanidae: (2) female *Pelecanus crispus;* (3) male *P. occidentalis;* (4) male *P. onocrotalus* (largest chromosome pairs only).



Figs. 5–6. Representative karyogrammes of Sulidae and Phalacrocoracidae: (5) male *Morus bassanus;* the inset shows the members of pair 1p in association in their original position in a metaphase plate (top; note secondary constriction approximately one third the length of the long arm) and the sex chromosome pair of a female (bottom); (6) male *Phalacrocorax carbo* Z-chromosome pair not identified).

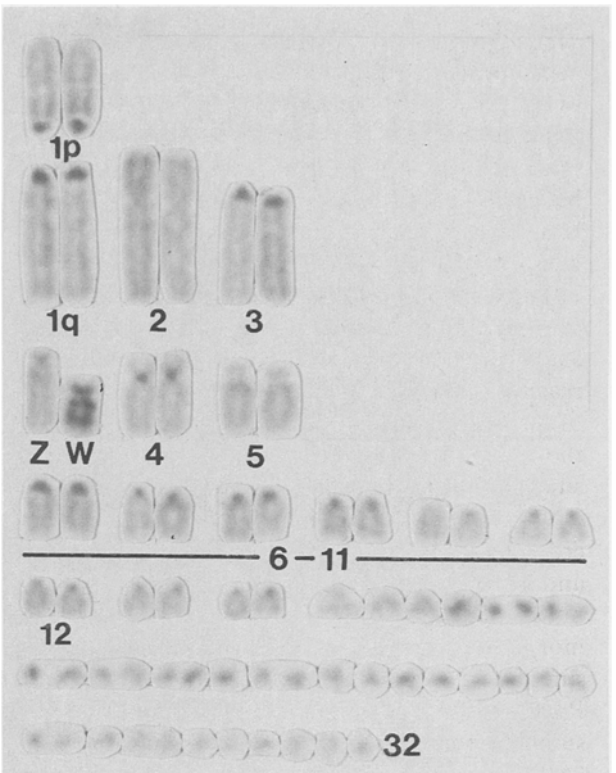


Fig. 7. C-banded karyogramme of *Morus bassanus* (Sulidae).

1q is much greater than that of the pelican chromosome 1 (Fig. 7). Thus, if pair 1 of the pelicans and pairs 1p and 1q of *M. bassanus* are related through centric fission or fusion, the addition or loss respectively of centromeric heterochromatin must have occurred. Pair 2 of *M. bassanus* is somewhat shorter than pair 2 of the pelicans. Since its arm ratio (2.2) is higher than in the pelicans (1.5, the value that is also found in many other birds with comparable morphology of the first three pairs; see above) a part of the short arm may be missing. As in the pelicans (and many other birds) pair 2 lacks distinctive centromeric heterochromatin (Fig. 7). Pair 3 is probably homologous to pair 3 of the pelicans, but lacks the characteristic minute short arm. There are two pairs of subtelocentric medium-sized macrochromosomes (pairs 4 and 5). The first of these is similar to pair 4 of the pelicans. Pair 5, which bears only minute short arms, is of the length of the long arm of pair 5 in the pelicans. The remaining autosomes are small to minute acrocentrics, gradually decreasing in size. This group may include the homologues of the short arm of the

pelican pair 5, those of the long and short arm of the pelican pair 6 (related through centric fusion/fission) and those of the biarmed pelican pairs 7 to 12 related through pericentric inversion probably, since there is little difference in diploid numbers between the pelicans and *Morus bassanus*). The W is a small submetacentric, darkly staining by C-banding (Fig. 7).

Thus, the karyotypes of the pelicans and of *Morus bassanus* differ in a considerable number of chromosome pairs, a difference for which at least 13 rearrangements would be responsible. The tracing of the exact nature of these rearrangements and of the exact homologies would be an interesting subject of detailed banding studies.

The karyotype of *Phalacrocorax carbo* (Phalacrocoracidae) differs from both the Pelecanidae and Sulidae karyotypes (Fig. 6). It has a diploid number of approximately 70. Pair 1 is of the same morphology as that in *Pelecanus*. Pair 2 is also similar, but has a slightly higher arm ratio (1.8). Pair 3 is of the same size as in the pelicans, but is submetacentric (A.R. 1.9) instead of subtelocentric A.R. 6.0 in *Pelecanus* spp.). There are six pairs of medium-sized biarmed chromosomes which are metacentric to submetacentric, except for one of the longest that is subtelocentric. This group probably includes the ZZ chromosome pair (only a male specimen was studied). Apart from the largest metacentric pair which is similar to pair 6 of the pelicans, this group does not show elements that are morphologically identical to chromosomes of *Pelecanus* or *Morus*. Except for two pairs of small submetacentrics (pairs 10 and 11) the remaining autosomes of *P. carbo* are small to minute acrocentrics. The karyotype of *P. carbo* has been previously studied by Oguma (1937), but a comparison with his data is not justified because of the completely different techniques used.

The karyotype of *Phalacrocorax carbo* presented here differs greatly from that of *Halietor* (=*Phalacrocorax*) *pygmaeus* described by Theodorescu 1975 . This author reported a diploid number of 70 and a karyotype entirely made up of acrocentrics, except for a metacentric Z. A similar karyotype was described for *Halietor* (=*Phalacrocorax*) *niger* by Patnaik & Samanta (1981) who, however, reported a diploid number of 90 for this species. Strange enough, Patnaik *et al.* stated that *Phala rocorax carbo* was very similar in karyotypic morphology to *H. niger*, based on inaccessible observations of Patnaik (1978). This observation is in clear disagreement with ours.

So far the karyology of the Pelecaniformes is rather poorly documented. The scarce data available indicate that, while the Pelecanidae form a homogeneous group, the order as a whole is very heterogeneous and forms an attractive subject for studies on interfamiliar karyological relationships including the as yet unstudied families Fregatidae, Anhingidae and Phaetonidae.

Ciconiiformes

Recently, a review of the karyology of the Ciconiiformes was provided by De Boer & Van Brink (1982). Here we confine ourselves to the presentation of some additional data.

In the family Ardeidae the karyotype of *Ardea goliath* (2n = 68) (Fig. 8) is nearly identical to the karyotypes of *A. cinerea* and *A. purpurea* (Ray-Chaudhuri, 1976; Mori, 1968; Itoh *et al.*, 1969; Hammar, 1970; Klein, 1973). The only possible exception is that the W of *A. goliath* is acrocentric instead of subtelocentric. The species shares the characteristic submetacentric morphology of pair 3 with all other ardeids studied so far.

In the family Ciconiidae, the karyotype of *Leptoptilos javanicus* (Fig. 9) is found to be nearly

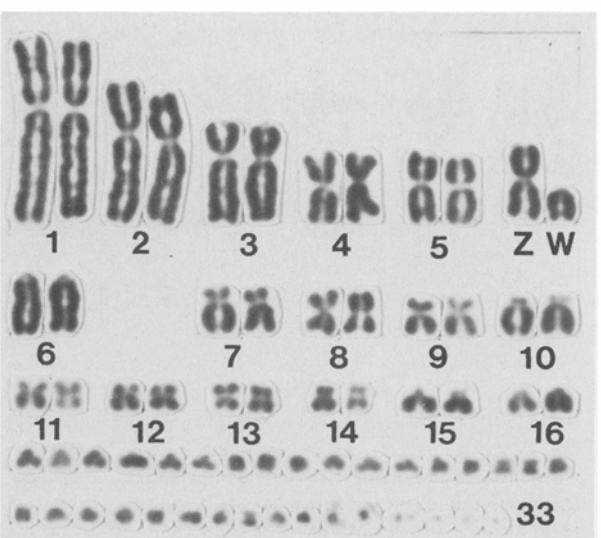
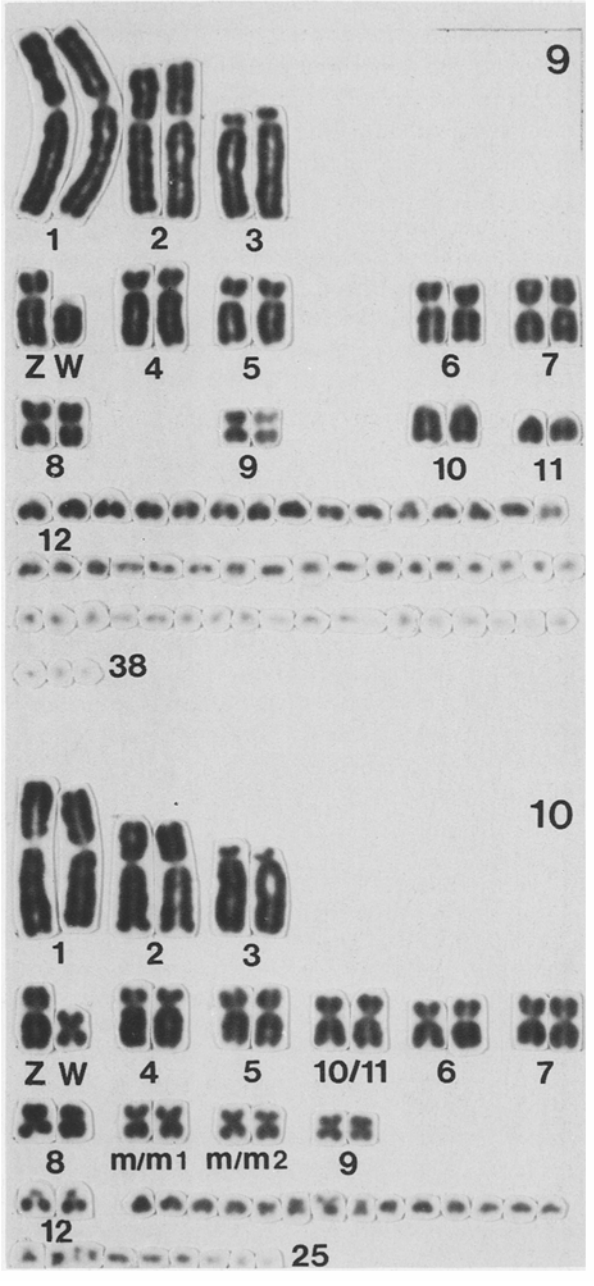


Fig. 8. Representative karyogramme of female *Ardea goliath* (Ardeidae).



Figs. 9–10. Representative karyogrammes of Ciconiidae: (9) female *Leptoptilos javanicus;* (10) female *L. crumeniferus.* Chromosome numbering in the latter is based on the supposition that an additional pair of submetacentrics (pair 10/11) evolved from fusion between acrocentric pairs 10 and 11 of the former species, while two additional pairs of small metacentrics (pairs m/m1 and m/m2) evolved from fusions between microchromosomes.

identical to those of *Ciconia ciconia, C. maguari, Ephippiorhynchus asiaticus* and *E. senegalensis* (De Boer & Van Brink, 1982). It shares the morphology of pairs 1 to 3 with all hitherto studied Ciconiidae and 7 additional pairs of individually recognizable autosome pairs (pairs 4 and 5 subtelocentrics, pair 6 submetacentrics, pairs 7–9 metacentrics of medium to small size, pair 10 acrocentrics twice the size of the next smaller pair of autosomes). The remaining elements are small to minute acrocentric microchromosomes. The diploid number is slightly higher than those of the four species mentioned above (78 versus 66–72). The Z chromosome is a submetacentric and the same size as pair 4; the W is acrocentric and the same size as pair 10.

This finding is rather surprising as De Boer & Van Brink (1982) reported *Leptoptilos crumeniferus* to possess a clearly different set of chromosomes. In Figure 10 the karyotype of the latter species is shown for comparison. In this illustration an attempt is made to number the chromosome pairs of *L. crumeniferus* according to supposed homologies with *L. javanicus. L. crumeniferus* has a diploid number of only 52. It lacks the acrocentrics of pair 10, but shows an additional pair of submetacentrics, the long arm of which corresponds in length with pair 10 in *L. javanicus.* It is possible that this pair fused with a pair of microchromosomes (possibly the largest, pair 11) and thus, the additional submetacentrics are numbered pair '10/11' in Figure 10. There are two additional pairs of small metacentrics in *L. crumeniferus.* Since these are clearly larger than the largest microchromosomes in *L. javanicus* they probably evolved from fusions between smaller microchromosomes. They are therefore numbered pairs 'm/m1' and 'm/m2'. The largest microchromosomes of *L. crumeniferus* bear a characteristic secondary constriction near the centromere (pair 12). There are, including pair 12, only 26 microchromosomes (versus 56 in *L. javanicus*). Since, as far as can be detected only three chromosome fusions are involved in the karyotypic differences between both species (accounting for a chromosome number difference of 6) it remains largely unclear which mechanism caused the great difference in diploid numbers. Apart from the autosomal differences the W-chromosome of *L. crumeniferus* is metacentric instead of acrocentric in *L. javanicus.*

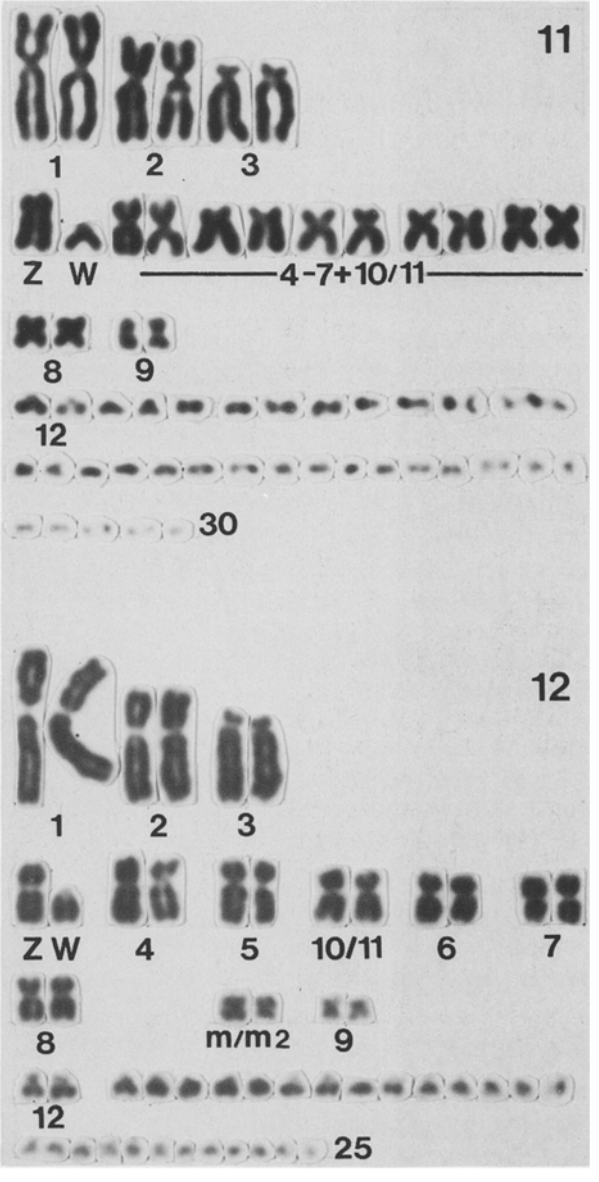
It is even more surprising that the karyotype of *Ciconia nigra* closely resembles that of *Leptoptilos crumeniferus* (De Boer & Van Brink, 1982). The chromosome complement of these species proved to be nearly identical on reinvestigation (Fig. 12). The diploid number of *C. nigra* is also 52, the acrocentrics of pair 10 are also absent and instead the pair 10/11 is found, while the largest microchromosomes (pair 12) bear a secondary constriction as in *L. crumeniferus*. The only difference is that *C. nigra* has one instead of two additional pairs of small metacentrics (pair m/m2). The W-chromosome of *C. nigra* is acrocentric.

The karyotype of *Ciconia* (=*Dissoura*) *episcopus* shows a resemblance with that of *C. nigra*. In both pair 10 is absent and they share the presence of pair 10/11. The chromosome number, however, is not as low (2n = approx. 62) and the small metacentrics of pairs m/m1 and m/m2 were not found. Since the material from this species available is of rather poor quality (Fig. 11), the latter cannot be stated with certainty.

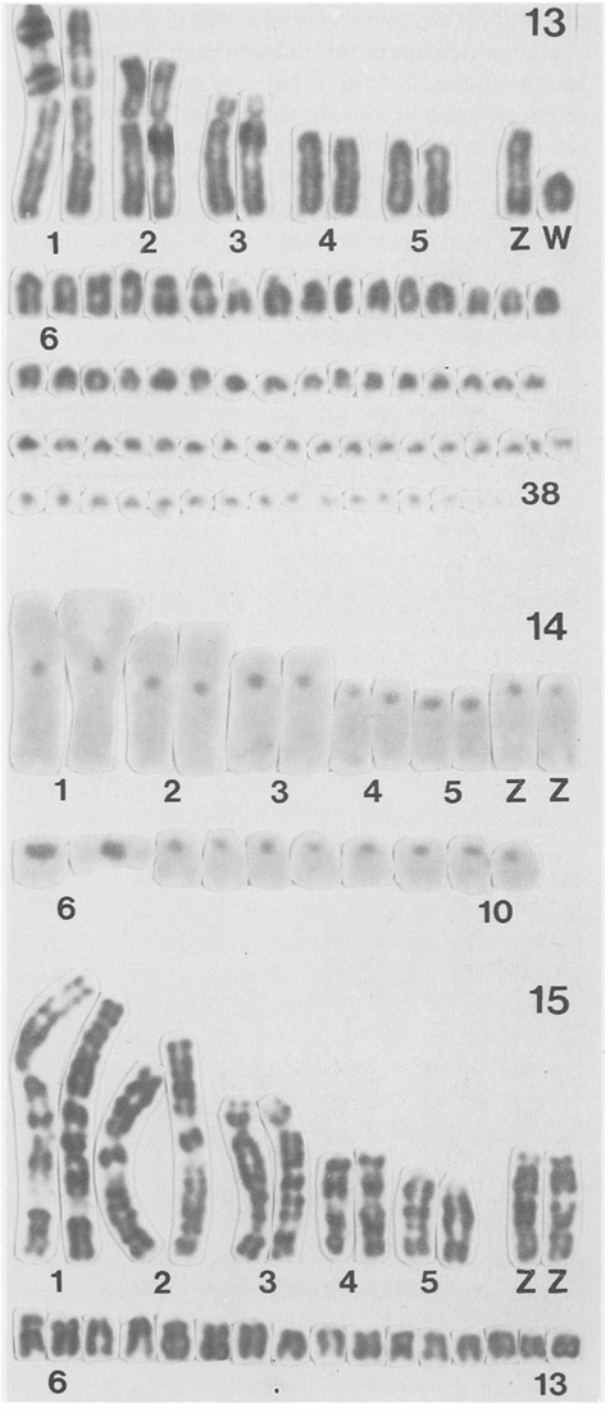
Since karyotypes with high diploid numbers (66–78) and identical macrochromosome morphology are found in five species of three ciconiid genera, viz. *Ciconia ciconia, C.* (=*Euxenura*) *maguari, Ephippiorhynchus senegalensis, E.* (=*Xenorhynchus*) *asiaticus* and *Leptoptilos javanicus,* it is tempting to suppose that this karyotype is ancestral to the family. In that case, it can hardly be imagined that the nearly identical karyotypes of *Leptoptilos crumeniferus* and *Ciconia nigra* evolved independently. If, however, they had a common origin (an origin possibly also shared in part by *C. episcopus*) this would have important taxonomic implications. Thus, the Ciconiidae constitute an ideal subject for further detailed karyological studies. In addition, the mechanism that caused the great reduction of the diploid number poses an interesting problem.

Anseriformes

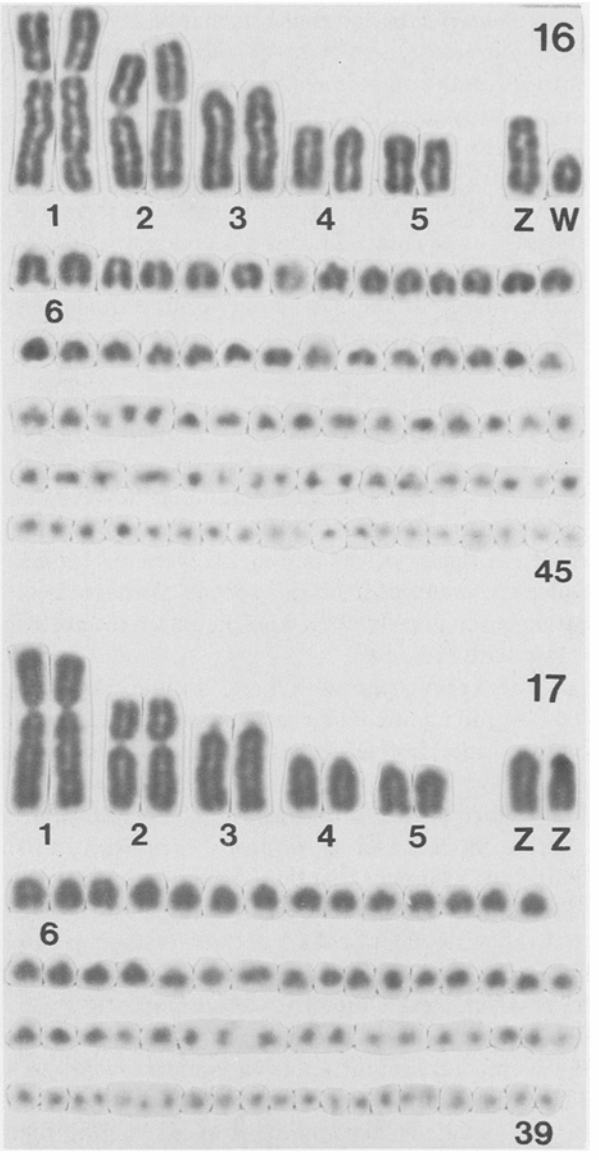
Five species of Anseriformes new to cytology were studied, viz. *Anseranas semipalmata* (Anatidae, Anseranatinae), *Cereopsis novaehollandiae* (Anatidae, Anserinae, tribe Anserini), *Chloephaga rubidiceps* (Anatidae, Anatinae, tribe Tadornini), *Anas castanea* (Anatidae, Anatinae, tribe Anatini) and *Netta rufina* (Anatidae, Anatinae, tribe Aythyini). All five have nearly identical karyotypes (Figs. 13–19) with diploid numbers of 80 or just above. There are three pairs of large macrochromosomes, which are of the characteristic morphology found in the first three pairs of many bird taxa (compare the karyotypes of Figs. 4–6 and 9–12 and see discussion on Pelecanidae). Pair 3, however,



Figs. 11–12. Representative karyogrammes of Ciconiidae: (11) female *Ciconia* (=*Dissoura*) *episcopus* (the relatively poor quality of this plate did not allow the determination of the exact number of small metacentrics); (12) female *C. nigra* (3 500×). In both species the chromosome pairs are numbered according to the nomenclature used in *Leptoptilos crumeniferus* (Fig. 10).

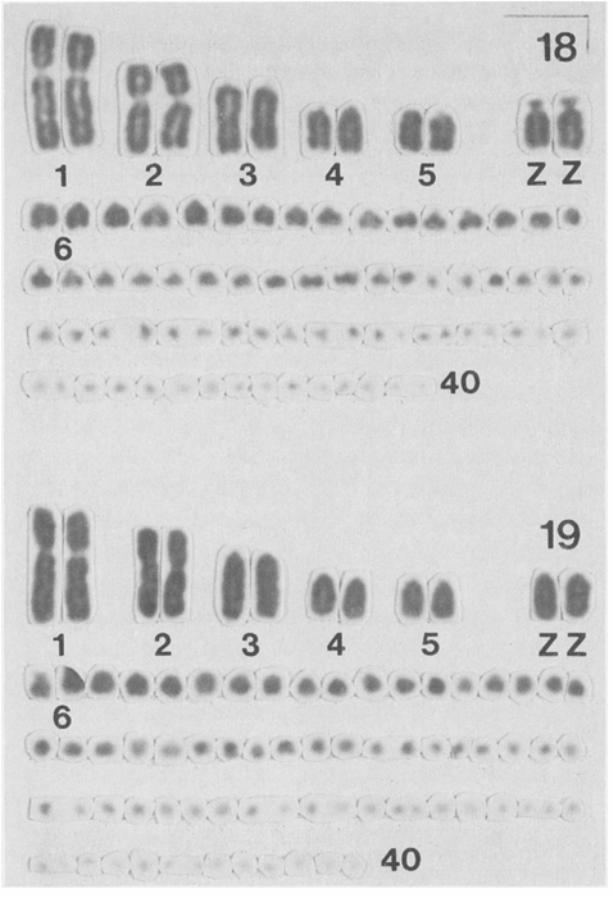


Figs. 13–15. Representative karyogrammes of *Anseranas semipalmata* (Anatidae): (13) orcein stain (female); (14) C-banding (male; first 10 pairs and sex chromosomes only); (15) BUdR-R-banding (male, first 13 pairs and sex chromosomes only).



Figs. 16–17. Representative karyogrammes of Anatidae: (16) female *Cereopsis novaehollandiae;* (17) male *Chloephaga rubidiceps.*

shows some variation: it is subtelocentric in *Anseranas semipalmata* (as in many other bird orders), but lacks the small short arms in the other four species. Pairs 4 and 5 are of medium size and acrocentric. Pair 6 is approximately half the size of pair 5, and from no. 6 on there is a gradual decrease in size. All the microchromosomes are acrocentric or of uncertain centromeric position. The Z-chro-

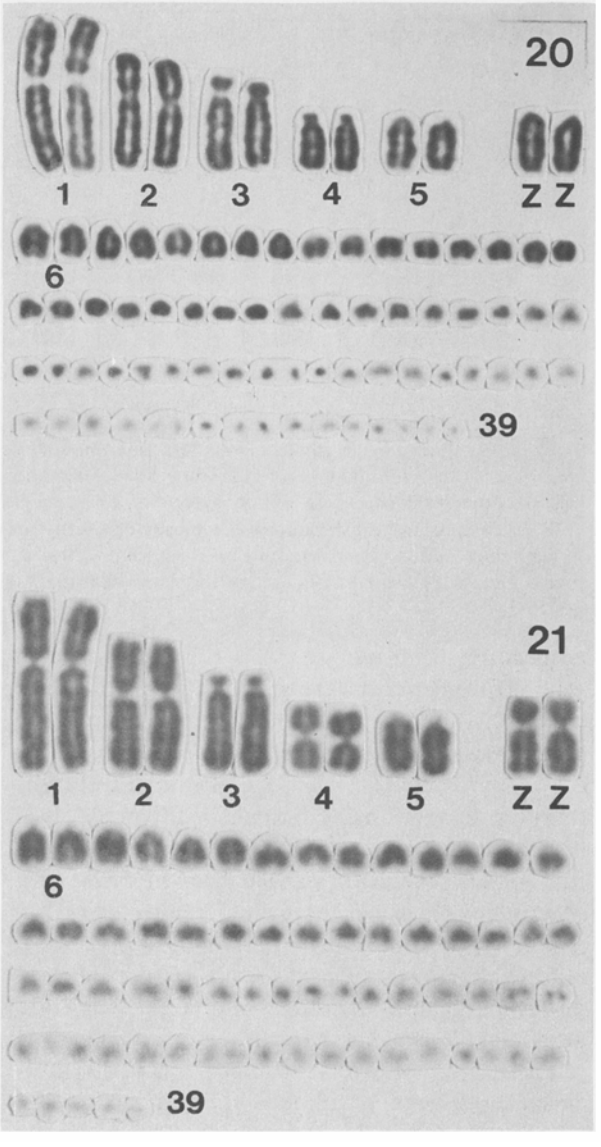


Figs. 18–19. Representative karyogrammes of Anatidae: (18) male *Anas castanea;* (19) male *Netta rufina.*

mosome is acrocentric (except in *Anas castanea* where it bears minute short arms), slightly larger than the autosomes of pair 4; the W is also acrocentric and a little longer than the autosomes of pair 7 as shown by C-banding (see Fig. 14).

This karyotypic structure is very common among the Anseriformes. Table 2 gives a summary of the karyotaxonomy of this order based on the karyotypes of 41 species (including the above five). It seems very likely that the karyotypic morphology described above is ancestral to the Anseriformes, since it is found in the families Anhimidae and Anatidae, as well as in representatives of each of the subfamilies and tribes of the latter. A problem is posed by the exact structure of pair 3, which may either be acrocentric or possess short arms of variable length. Since very distinct short arms are found in *Chauna chavaria* (Fig. 20) and *Anseranas semipalmata,* members of Anhimidae and Anatidae respectively, it seems likely that a subtelocentric pair 3 was original, the more so since the subtelocentric morphology is also found in other orders with comparable pairs 1–3 (see discussion on Pelecaniformes). Most of the Anatidae lack the short arms in pair 3 or show smaller short arm regions (like in *Anser cygnoides,* Fig. 21).

Only in four genera are clearly different karyotypes found (for all relevant references see De Boer, 1984). In *Branta* (Anatidae, Anserinae, Anserini) the Z-chromosome is submetacentric instead of



Figs. 20–21. Representative karyogrammes of: (20) male *Chauna chavaria* (Anhimidae); (21) male *Anser cygnoides* (Anatidae).

Table 2. Karyotaxonomy of 41 species of Anseriformes. This list indicates the presence of the supposed original anseriform karyotype (see text) in the various taxa of the order and the karyotypic changes that are found in several individual species.*

Family	Subfamily	Tribe	Genera studied (no. species)	Original karyotype (no. species)	Karyotypic changes (no. species)
ANHIMIDAE			*Chauna* (1)	× (1)	
ANATIDAE	Anseranatinae		*Anseranas* (1)	× (1)	
	Anserinae	Dendrocygnini	*Dendrocygna* (1)	× (1)	
		Anserini	*Cygnus* (2)	× (2)	
			Branta (1)		smZ (1)
			Anser (9)		m4, smZ (5) a2, sm4, smZ (4)
			Cereopsis (1)	× (1)	
		Stictonettini	–		
	Anatinae	Tadornini	*Chloephaga* (1)	× (1)	
			Apolochen (1)	× (1)	
			Tadorna (1)	× (1)	
		Tachyerini	–		
		Cairini	*Cairina* (1)	× (1)	
			Nettapus (1)	× (1)	
			Aix (2)		a1, a2 (1) high A.R. 1 (1)
		Anatini	*Anas* (7)	× (7)	
		Aythyini	*Netta* (1)	× (1)	
			Aythya (5)	× (5)	
		Mergini	*Somateria* (1)	× (1)	
			Bucephala (1)		st1–5, stZ (1)
			Mergus (2)	× (2)	
		Oxyurini	*Oxyura* (1)	× (1)	

* Two species studied by De Aguiar (1968), viz. *Amazonetta brasiliensis* and *Dendrocygna viduata,* are not included because of lack of precise information on karyotypic structure. Species recorded in the older literature, which were studied from sectioned gonadal material, are excluded as well. For complete sets of references see De Boer, 1984.
The last column gives the structure of chromosome pairs that are different from corresponding pairs in the supposed original karyotype (sm = submetacentric, m = metacentric, a = acrocentric, st = subtelocentric). Slight differences in the length of the short arm regions of pair 3 and the Z-chromosomes are not taken into consideration.

acrocentric. In *Anser* the Z is submetacentric as well, while in part of its species pair 4 is metacentric (see Fig. 21 for comparison), whereas the remaining species possess a submetacentric pair 4 and an acrocentric pair 1. In *Aix* (Anatidae, Anserinae, Cairini) there are also two forms, one which differs only by a higher arm-ratio of pair 1, another in which both pair 1 and pair 2 are acrocentric. In *Bucephala* (Anatidae, Anatinae, Mergini), finally, pairs 1–5 and the Z-chromosome are subtelocentrics with minute short arms. Apart from these four clearly divergent genera there is only minor variation among the other Anseriformes with regard to the relative length of the Z-chromosome and the presence or absence of minute short arms on the chromosomes of pairs 3, 4 and 5.

Because of the relatively high karyotypic uniformity of the order and the presence of only a few divergent karyotypes in different tribes of the Anserinae and Anatidae, so far no phylogenetic conclusions can be drawn from the karyological data. As long as no additional information on a broader series of species is obtained it must be assumed that the divergent karyotypes of *Bucephala, Aix* and *Anser* envolved independently from the original Anseriform karyotype. The karyotype of *Branta,* which shares the submetacentric Z with *Anser,* may have had a common origin.

The karyotypic structure most common among the Anseriformes and most probably ancestral to the order is also found in most representatives of the Ratitae with the possible exception of the structure of the W-chromosome. Thus, we may be dealing with one of the most primitive avian karyotypes (Takagi *et al.,* 1972; Takagi & Sasaki, 1974; De Boer, 1980).

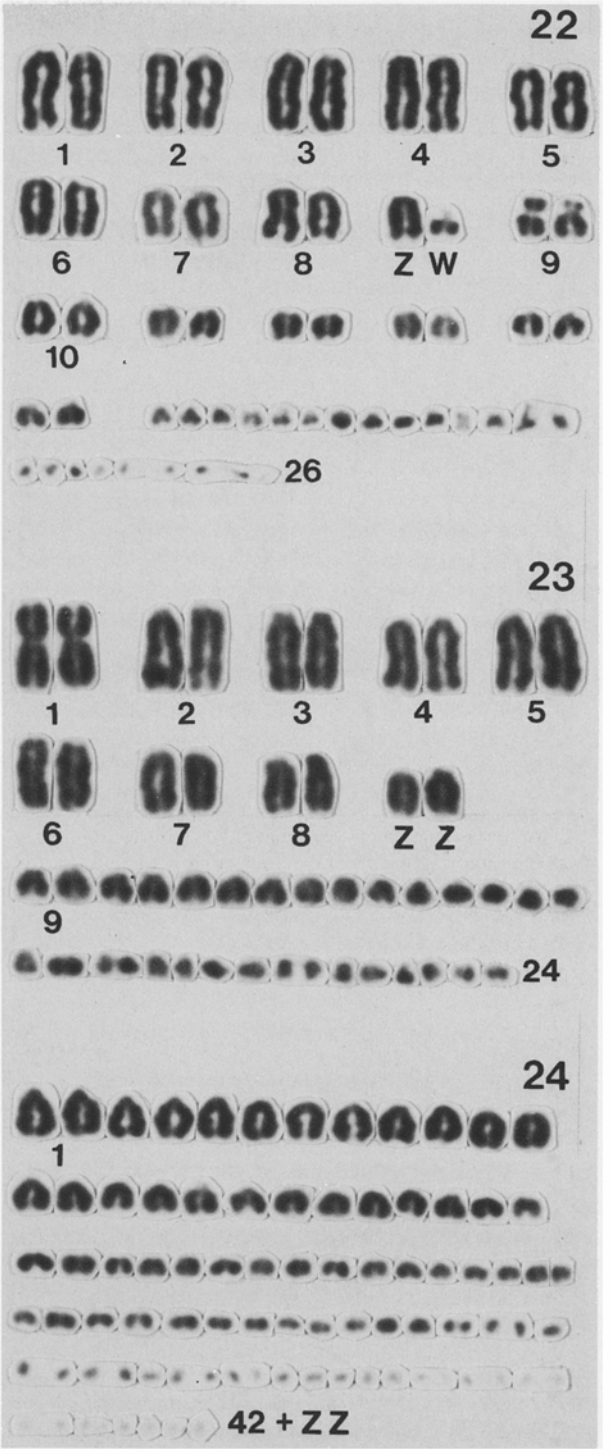
Falconiformes

An account on the karyological heterogeneity of the Falconiformes was given by De Boer (1975, 1976), while the karyotypes of 16 new species of the family Accipitridae are described and discussed by De Boer & Sinoo, 1984. Here, only some new data on three species of the Falconidae are presented.

The karyotype of *Falco jugger* (Fig. 23), first described here, is identical to that of *F. sparverius* (Shoffner, 1974; De Lucca, 1983). It has a diploid number of 48, and apart from the metacentrics of pair 1 all autosomes are acrocentric. There is a clear drop in chromosome length between pairs 8 and 9. The Z is acrocentric, somewhat smaller than pairs 7 and 8, but not clearly recognizable in unbanded material. The W probably is a small acrocentric.

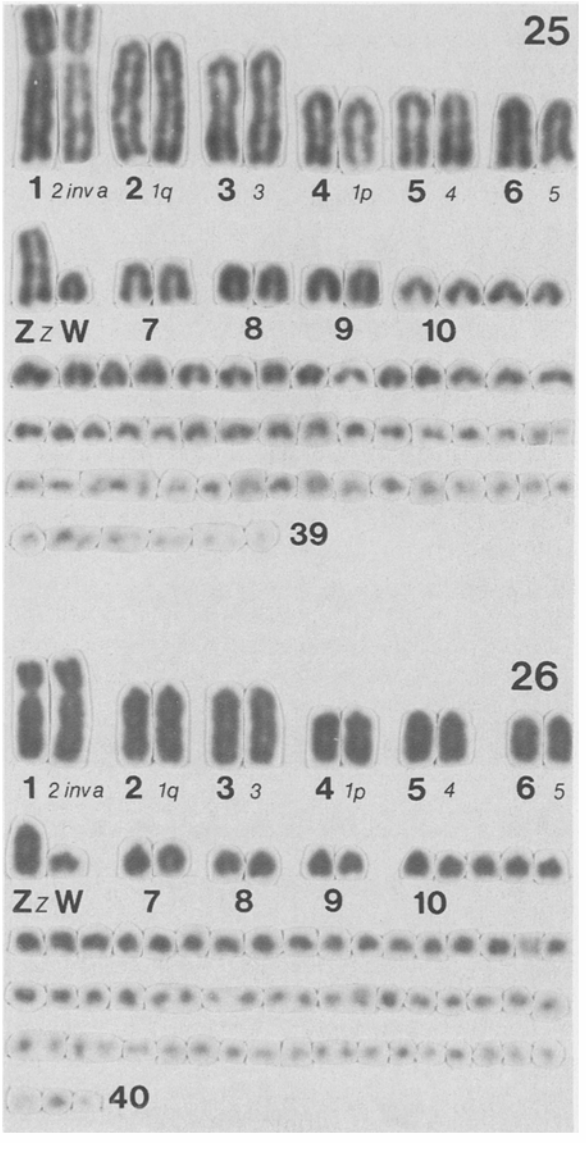
The chromosomes of *Falco biarmicus* were described by De Boer (1976). They are shown for comparison in Figure 22. In common with *F. tinnunculus* (Renzoni & Vegni-Talluri, 1966; Bulatova, 1977) and *F. chiquera* (Kaul & Ansari, 1976) it lacks the metacentric pair 1 found in *F. jugger* and *F. sparverius*. Instead, in the former three falcons there is one additional pair of equal sized acrocentrics, possibly related through pericentric inversion. In addition they possess a slightly higher diploid number (approx. 52) because of the presence of 4 additional small to minute chromosomes. The sex chromosomes of all falcons are similar. *Falco biarmicus* and *F. chiquera* differ from *F. tinnunculus* by the presence of short arms in pair 9, which is acrocentric in the latter species. This is also true for *F. jugger* and *F. sparverius*. One of the *F. biarmicus* specimens we studied showed structural heterozygosity in pair 9, one of the homologues being submetacentric, the other subtelocentric. The other two specimens were homozygous for the submetacentric variant.

The karyotype of *Milvago chimachima* (Fig. 24) differs clearly from those of *Falco* and shows close resemblance to that of *Polyborus plancus* (De Boer, 1976). It has a diploid number of approximately 84 and the complement is exclusively made up of acrocentrics of medium to small size. Because of the very gradual decrease in size, the chromosomes could not be arranged in pairs, nor could the possible sex chromosomes be identified. This karyotype differs only in one respect from that of *P. plancus*, viz. in the absence of a pair of medium-sized metacentrics. These metacentrics in *P. plancus* are clear-



Figs. 22–24. Representative karyogrammes of Falconidae: (22) female *Falco biarmicus* (note slightly heteromorphic pair 9); (23) male *F. jugger* (note absence of biarmed pair 9 and median centromeric position of pair 1); (24) *Milvago chimachima* of unknown sex (chromosomes not arranged in pairs because of insufficient distinction).

ly smaller than those of pair 1 in *Falco jugger* and *F. sparverius,* but larger than the submetacentrics of pair 9 in *F. biarmicus* and *F. chiquera.* Thus, these three types of biarmed chromosomes are probably not homologous and evolved independently. Since for the remainder all Falconidae exclusively show acrocentric chromosomes it remains unclear which mechanism caused the great difference in diploid numbers between *Falco* on the one hand and *Milvago* and *Polyborus* on the other.



Figs. 25–26. Representative karyogrammes of Megapodiidae: (25) female *Aepypodius arfakianus;* (26) female *A. bruijnii.* For explanation of additional numbering of macrochromosomes in italics see Figure 35.

Galliformes

The karyotypes of seven species of gallinaceous birds new to cytology were studied: *Aepypodius arfakianus* and *A. bruijnii* (Megapodiidae), *Ortalis canicollis* (Cracidae), *Lophura edwardsi* and *L. imperialis* (Phasianidae) and *Guttera plumifera* and *G. edouardi* (Numididae).

The karyotypes of the two megapodes, *Aepypodius arfakianus* (Fig. 25; G-banding in Fig. 34) and *A. bruijnii* (Fig. 26) are identical. Their diploid numbers are approximately 80. There are six pairs of macro-autosomes: pair 1 is submetacentric (A.R. 2.3), pairs 2 and 3 are large acrocentrics and pairs 4–6 are acrocentrics of medium size. All remaining autosomes are acrocentric or of unidentifiable centromeric position. The chromosomes of pair 7 are approximately half the size of those of pair 6, and from there on there is a very gradual decrease of size. The Z-chromosome is acrocentric, of the size of pair 4. Only one single other megapode has been studied, *Macrocephalon maleo* (Sasaki *et al.,* 1982) but so far no detailed information on its karyotypic structure is available.

The karyotype of the cracid *Ortalis canicollis* (Fig. 27) is identical to those of *Crax mitu* (De Boer & Belterman, 1981; Takagi & Sasaki, 1974) and *Penelope jacquacu* (Sasaki *et al.,* 1982) of the same family. *Crax mitu,* however, was found to possess a higher diploid number (approx. 100 compared to 78–80 in the other two), due to the presence

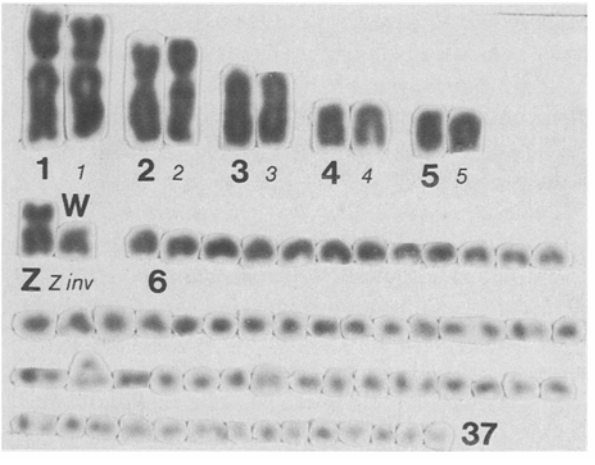
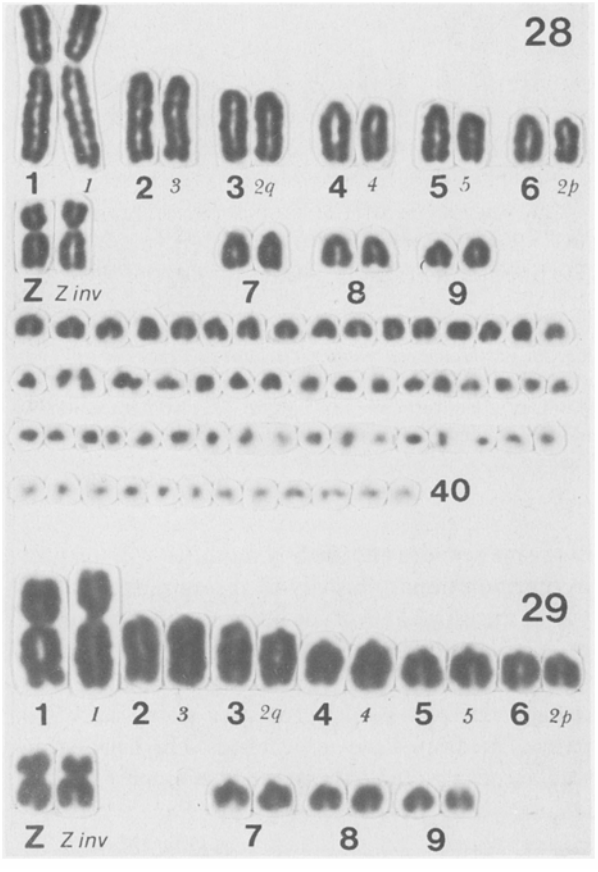


Fig. 27. Representative karyogramme of female *Ortalis canicollis* (Cracidae) (3 500×). For explanation of additional numbering of macrochromosomes in italics see Figure 35.

of many more minute microchromosomes. The first three pairs of macrochromosomes are of the characteristic morphology found in many other avian taxa (see discussion on Pelecaniformes), except for the absence of short arms in pair 3. Pairs 4 and 5 are medium-sized acrocentrics. The remaining autosomes are small to minute and as far as detectable, of acrocentric morphology. The Z is a submetacentric somewhat larger than pair 4; the W is a small submetacentric.

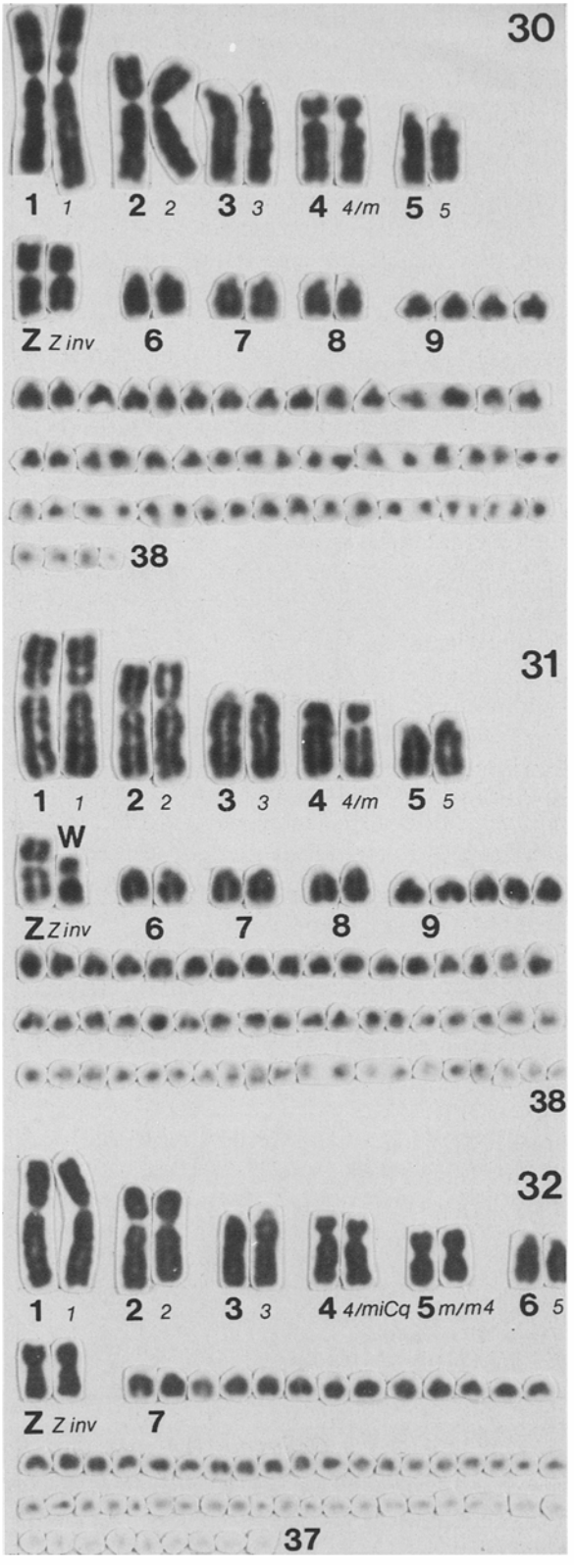
The karyotypes of the two pheasants, *Lophura edwardsi* (Fig. 28) and *L. imperialis* (Fig. 29) are identical to those of all other pheasants of the genera *Lophura, Phasianus, Chrysolophus, Syrmaticus* and *Polyplectron* studied to date (for a complete set of references see De Boer, 1984). Their diploid numbers are approximately 82. Pair 2 of the cracidae apparently is absent. Two additional pairs of medium-sized acrocentrics are found instead. Since the smallest of these is intermediate in size between macro- and microchromosomes there is a less clear boundary between these groups than in the cracids.



Figs. 28–29. Representative karyogrammes of Phasianidae s.s.: (28) male *Lophura edwardsi;* (29) male *L. imperialis* (largest chromosomes only; magnification 3 500× instead of 3 000× in other illustrations). For explanation of additional numbering of macrochromosomes in italics see Figure 35.

The karyotypes of the numidids *Guttera plumifera* (Fig. 30) and *G. edouardi* (Fig. 31) differ from those of the two members of the Numididae hitherto studied, *Acryllium vulturinum* and *Numida meleagris* (for references see De Boer, 1984). *N. meleagris* was found to possess a unique pair of medium-sized metacentric autosomes (Stock & Bunch, 1982). Judging from unbanded material this pair is also present in *A. vulturinum* (Takahashi & Hirai, 1974; this report, Fig. 32). This pair is absent, however, in the karyotypes of both *G. plumifera* and *G. edouardi.* C-banding on the material of *G. plumifera* did not reveal the presence of an interstitional C-band in the long arm of pair 4, which was described as a characteristic of *N. meleagris* by Stock & Bunch (1982). Otherwise the karyotypes of the four numidids are identical. Both specimens of *G. plumifera* studied showed structural heterozygosity for pair 6, one of the homologues being slightly longer than the other. The karyotypes of *G. plumifera* and *G. edouardi* differ from the cracid karyotypes mainly by the presence of short arms on the chromosomes of pair 4. They also closely resemble the karyotype of *Gallus domesticus* (Fig. 33), which differs only by the shorter p arm of pair 4, the more metacentric Z and the presence of short arms in two of the largest microchromosomes (pairs 6 and 7).

Thanks mainly to the detailed chromosome banding analysis of 8 gallinaceous birds by Stock & Bunch (1982), one of the best examples of the use of banding studies in avian karyotaxonomy, we now have a reasonably good picture of the major evolutionary karyotypic changes in the order. This picture was supplemented by the banding work on *Crax mitu* by De Boer & Belterman (1981), on *Penelope jaquacu* by Sasaki *et al.* (1982), on *Coturnix coturnix* and *Gallus domesticus* by Sasaki (1981), on *C. coturnix, G. domesticus* and *Meleagris gallopavo* by Ryttman & Tegelström (1981a), and that on birds of various orders by Takagi & Sasaki (1974). The data in the present communication, although largely based on unbanded material, supplement the picture with regard to the position



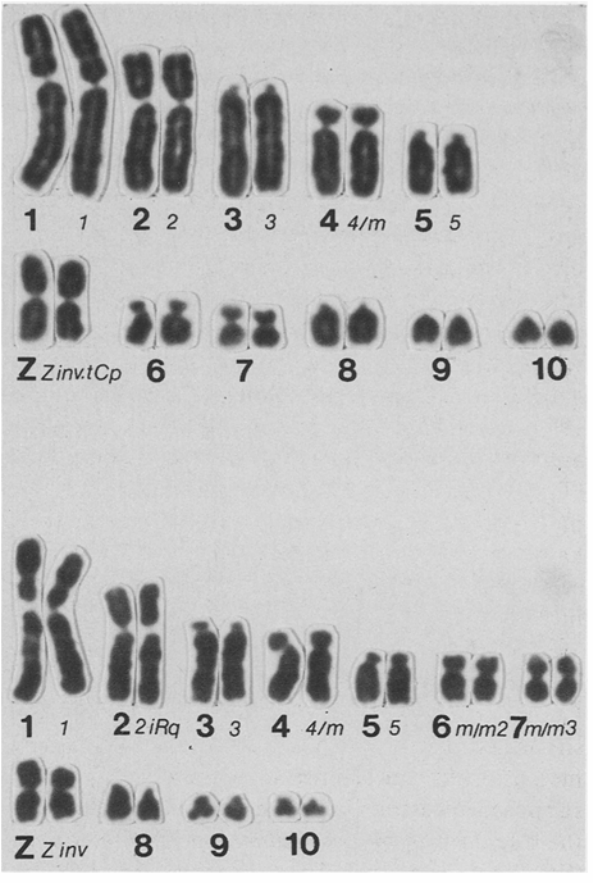


Fig. 33. Partial karyogrammes of *Gallus domesticus* (top) and *Afropavo congensis* (bottom) for comparison with the karyogrammes of galliform birds in Figs. 25–32. For explanation of additional numbering of macrochromosomes in italics see Figure 35.

of the megapodes and slightly modify it with respect to the evolutionary history of the numidids.

The ancestral karyotype of the Galliformes most probably included five pairs of macro-autosomes: a large metacentric pair 1, a somewhat smaller submetacentric pair 2, an acrocentric pair 3 and two pairs of medium-sized acrocentrics. The Z probably was acrocentric and the same size as pair 4. The

Figs. 30–32. Representative karyogrammes of Numididae: (30) male *Guttera plumifera* (note structural heterozygosity of pair 6); (31) female *G. edouardi;* (32) male *Acryllium vulturinum.* For explanation of additional numbering of macrochromosomes in italics see Figure 35.

remainder of the autosomes probably were acrocentrics of small to minute size, making a diploid number of approximately 80. This proposed ancestral karyotype was possibly very similar to that of most extant Anseriformes (see Figs. 13–19), which differ only from the above description by the presence of minute short arms in pair 3. In its turn, the anseriform karyotype is possibly very similar to the original avian karyotype. There are two reasons for this suggestion. Firstly, the anseriform karyotype closely resembles the karyotypes of the ratites, which means that it is represented in both avian subclasses, Carinatae and Ratitae (Takagi & Sasaki, 1974). Secondly, only minor differences are seen among the karyotypes of the various ratite groups (Takagi & Sasaki, 1972; De Boer, 1980), which means that we are dealing with a very ancient karyotype, since most ratite groups separated from each other early in the geological history of the birds.

Stock & Bunch (1982) suggested a possible karyological relationship between Galliformes and Cuculiformes. They based this suggestion on the similarity in the morphology of the acrocentric medium-sized autosomes of pairs 4 and 5 in the supposed ancestral Galliform karyotype and that of the cuculiform species *Gallirex porphyreolophus* (Stock & Mengden, 1975). As discussed previously (see Pelecaniformes), pairs 1, 2 and 3 of the ancestral galliform karyotype are found in various bird orders. However, chromosome banding data on the medium-sized chromosomes that are reliable enough to allow conclusions as to inter-order chromosome homology, are extremely rare. They are, for instance, not available for the ratites, the Anseriformes and most of the other orders. In the absence of such data for other orders the suggestion of a special relationship between Galliformes and Cuculiformes does not seem justified. For the time being, therefore we have based our above suggestion of a similarity between the supposed ancestral galliform karyotype with those of the Anseriformes and Ratitae on the gross morphology of the medium-sized chromosomes. Detailed banding analyses are certainly required to test this suggestion.

If we are right in the supposition that the original galliform karyotype closely resembled the ancestral avian karyotype, all extant gallinaceous species share at least one apomorphic character: the absence of short arms in pair 3 (see above). Stock & Mengden (1975) demonstrated that in *Gallus domesticus* the absence of these short arms can be explained as the result of a pericentric inversion (as compared to two species of Columbiformes). Thus, such an inversion may have preceded the first galliform speciation.

Although none of the extant galliform species so far studied possess the complete set of original chromosomes of the ancestral karyotype, all of them can be traced back in the various karyotypes. A brief account is given below of the karyotypes of the various galliform taxa in terms of the presence of original and derived chromosomes. A summary of the karyotypic changes that possibly led to the present karyological heterogeneity is given in Figure 35. In this illustration as well as in the text below a chromosome numbering system based on presumed homologies is used. This numbering often differs from that used in the above descriptions and illustrations of the individual karyotypes. To facilitate comparisons it is added in italics to the specific numbering in the karyotypes of Figures 25–34.

The two Megapodiidae studied here together with that reported by Sasaki *et al.* (1982) are unique among the Galliformes since they apparently lost the original metacentric pair 1 by a fissioning event, producing two pairs of acrocentrics (1p and 1q). All other groups retained the original pair 1, although in some lineages inversions occurred (Stock & Bunch, 1982). The megapodes, in addition, show a unique inversion in pair 2, resulting in a chromosome with a higher arm ratio than the original (see Fig. 34). Pairs 3, 4 and 5 are probably original in the megapodes, which have also retained the presumed original acrocentric Z.

All non-megapode galliform species studied to date have a biarmed Z-chromosome. However, metacentric, submetacentric as well as subtelocentric Zs are found. The submetacentric Z of the Cracidae and Numididae (De Boer & Belterman, 1981; Sasaki *et al.*, 1982; Stock & Bunch, 1982; this report) can be derived from the acrocentric megapode Z by pericentric inversion. The Z-chromosomes of the remaining species that have been banded *(Phasianus colchicus, Pavo cristatus, Colinus virginianus, Centrocercus urophasianus, Coturnix coturnix, Meleagris gallopavo, Gallus domesticus)* are nearly identical or can be derived from the submetacentric cracid-numidid Z by inversions and additions of heterochromatic material. Some of the unbanded

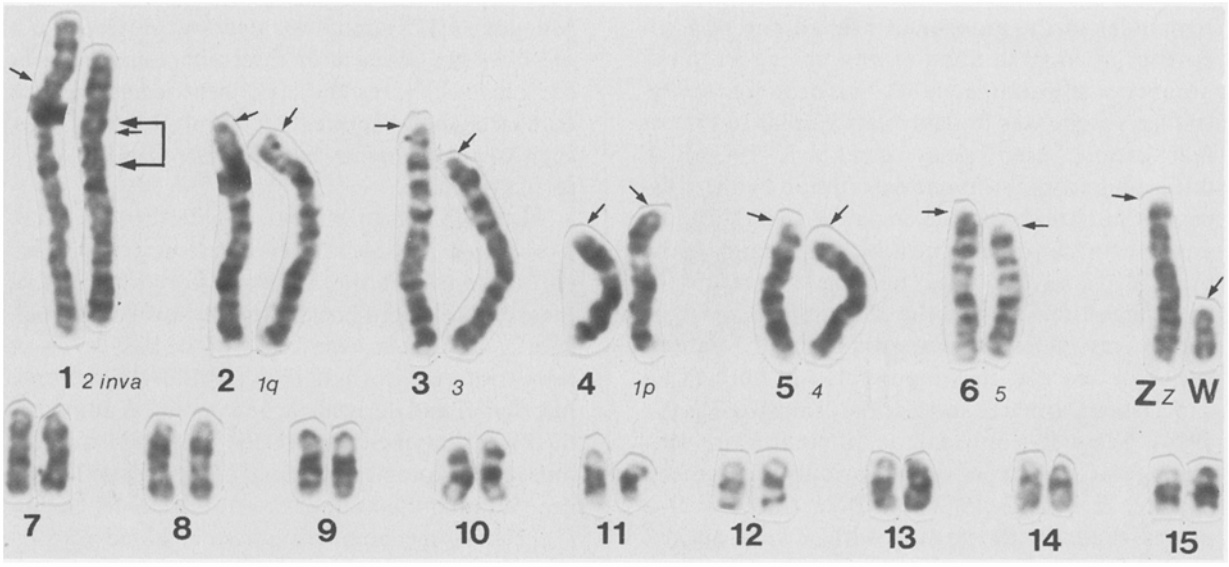


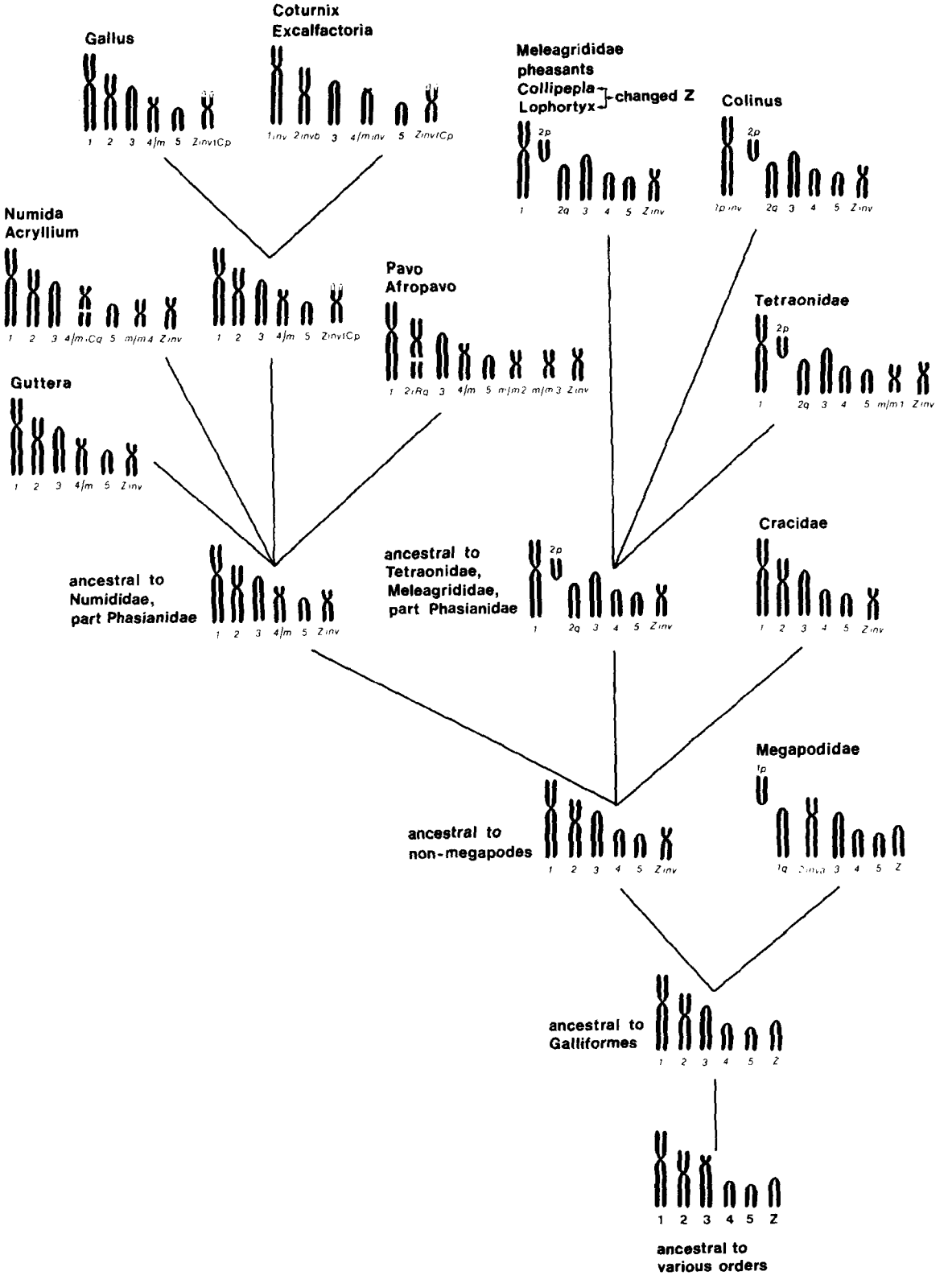
Fig. 34. Partial G-banded karyogramme of *Aepypodius arfakianus*. Small arrows point at centromeric positions of individual chromosomes. Large arrows in pair 1 delineate the pericentrically inverted section in this pair when compared to the homologous pair in the other galliform species. For explanation of additional numbering of macrochromosomes in italics see Figure 35.

karyotypes, however, show subtelocentric Z-chromosomes with minute short arms (e.g. *Collipepla squamata, Lophortyx gambelli* and *L. californica;* see Chromosome Atlas, 1971). Thus, it remains to be confirmed whether the possession of a non-acrocentric Z may be regarded as a synapomorphic character of all non-megapode galliformes.

The Cracidae show the supposed original pairs 1–5 and differ only from the supposed ancestral karyotype by the inverted submetacentric Z (De Boer & Belterman, 1981; Sasaki *et al.*, 1982).

According to Stock & Bunch (1982) *Phasianus* and *Colinus* (Phasianidae), *Meleagris* (Meleagrididae) and *Centrocercus* (Tetraonidae) share the loss of the original pair 2 by fissioning, resulting in the acrocentric pairs 2p and 2q. They retained the original pairs 1, 3, 4 and 5; judging from unbanded material presented here and elsewhere (for refer-

Fig. 35. Schematic representation of the possible evolutionary changes in the macrochromosomes of the Galliformes. This scheme is based on karyological evidence only (for full explanation and sources see text); no other characteristics are taken into consideration. Family names are used when all hitherto studied species share the macrochromosome morphology shown; whenever differences exist within families generic names are used. An attempt is made to use a uniform chromosome numbering system based on presumed interspecific homologies. In this nomenclature *1, 2, 3, 4, 5* and *Z* indicate the original macrochromosomes, while *1q* and *1p*, and *2q* and *2p* indicate the acrocentric products of the fissioned pairs *1* and *2; 4/m* indicates the product of a fusion between pair *4* and a microchromosome pair; *m/m1, m/m2, m/m3, m/m4* indicate the metacentric products of independent fusions of microchromosomes; *Zinv* and *1inv* indicate pericentrically inverted chromosomes *Z* and *1; 2inva* and *2invb* indicate two different pericentrically inverted pairs *2; 1pinv* indicates a pair *1* with a paracentric inversion in the short arm; *4/minv* indicates a pericentrically inverted pair *4/m; 4/miCq* indicates a pair *4/m* with an additional interstitional C-band in the long arm; *2iRq* indicates a pair *2* with an additional R-band (discovered as G-negative band by Stock & Bunch, 1982 in *Pavo;* not mentioned in the text) in the long arm; and *ZinvtCp* indicates a *Zinv* chromosome with an additional C-band in the short arm. This chromosome nomenclature is added in italics to the individual numbering systems for each species in the galliform karyogrammes of Figures 25–34. Some doubt exists with regard to the exact homology of the *Zinv* chromosome in the various genera. Especially in the group consisting of Meleagrididae, the pheasants (genera *Phasianus, Chrysolophus, Syrmaticus, Lophura* and *Polyplectron*), *Collipepla* and *Lophortyx* possible differences exist in the structure of this element that need to be studied in detail. The scheme does not express the possible differences in the length of the short arm of the *4/m* chromosome between *Gallus* and the Numididae (see text).


Gallus
Coturnix
Excalfactoria
Meleagrididae
pheasants
Collipepla
Lophortyx
changed Z
Colinus
Numida
Acryllium
Pavo
Afropavo
Tetraonidae
Guttera
ancestral to
Numididae,
part Phasianidae
ancestral to
Tetraonidae,
Meleagrididae,
part Phasianidae
Cracidae
Megapodidae
ancestral to
non-megapodes
ancestral to
Galliformes
1 2 3 4 5 Z
ancestral to
various orders

ences see De Boer, 1984) the same characteristics are met in many more Phasianidae: the other pheasants (genera *Chrysolophus, Syrmaticus, Lophura* and *Polyplectron*), *Collipepla* and *Lophortyx*. It is tempting to suggest that the fissioning of pair 2 occurred only once in the Galliformes, a suggestion which would combine part of the Phasianidae, Meleagrididae and Tetraonidae in one natural group. This view, adopted by Stock & Bunch (1982), however, should be confirmed since independent fissioning of pair 2 apparently occurred in many avian orders and families. Among the Galliformes with a fissioned pair 2, the Tetraonidae (*Centrocercus;* Stock & Bunch, 1982) stand apart because of the possession of a unique metacentric chromosome ('m/ m1'), which resulted from fusion of two microchromosome pairs. *Colinus* differs from the other banded species in this group by a paracentric inversion in the p arm of pair 1. Banding studies in other species, especially phasianids related to the pheasants, e.g. *Collipepla* and *Lophortyx* should be carried out to detect possible further rearrangements. In any case there appears to be variation as to the centromeric position of the Z-chromosome (see above).

According to Stock & Bunch (1982) part of the Phasianidae, viz. *Gallus domesticus, Pavo cristatus* and *Coturnix coturnix,* and the Numididae *(Numida meleagris)* share a submetacentric chromosome pair ('4/ m') which is the product of a fusion of the original pair 4 and a pair of microchromosomes. The presence of a submetacentric pair 4 is shared also by the numidids *Acryllium vulturinum* (Takahashi & Hirai, 1974; this report) and *Guttera plumifera* and *G. edouardi* (this report) and by the phasianid *Afropavo congensis* (De Boer & Van Bocxstaele, 1981). The short arms of the submetacentric pair 4, however, are clearly smaller in *Gallus* than in the other species (pers. obs.), while in *Numida meleagris* an additional interstitional C-band is found in the long arm (Stock & Bunch, 1982), not present in the other banded species. In addition, a pericentric inversion would have changed the morphology of this pair in *Coturnix coturnix* (Stock & Bunch, 1982) into an almost acrocentric chromosome, a characteristic probably shared by *Excalfactoria chinensis* (King, 1970) as judged from unbanded chromosomes. Thus, if the submetacentric pair 4/ m resulted from a unique fusion event, it underwent several independent changes afterwards which should be subjected to further detailed study.

All members of the group of species possessing the 4/ m chromosome pair retained the original pairs 1, 2, 3 and 5, except *Coturnix coturnix* (and possibly *Excalfactoria chinensis*) which shows pericentric inversions in pairs 1 and 2 (Stock & Bunch, 1982; Sasaki *et al.,* 1982). *Pavo cristatus* possesses two new pairs of medium-sized metacentrics ('m/ m2' and 'm/ m3') which originated from fusions between microchromosomes (Stock & Bunch, 1982). Judging from unbanded material, this characteristic is shared by *Pavo muticus* (Sasaki *et al.,* 1968) and *Afropavo congensis* (De Boer & Van Bocxstaele, 1981; see Fig. 33). In *Numida meleagris* Stock and Bunch found one additional pair of medium-sized metacentrics ('m/ m4') which appeared to have evolved independently from those in *Pavo,* by fusion of different microchromosomes. This characteristic is also found in *Acryllium vulturinum* (Takahashi & Hirai, 1974 compare Fig. 32), but not in *Guttera* (this report). Thus, m/ m4 must have evolved after the first speciation of the numidid stock. The presence of an additional C-band in the q arm of chromosome 4/ m probably is unique for *N. meleagris;* it is absent in *G. plumifera* (this report) and judging from the relative length of 4/ m it is not likely to be present in *A. vulturinum* (see Fig. 32).

In the group of species sharing the 4/ m chromosome pair, the karyotypes of *Guttera* probably resemble most closely the original karyotype. *Gallus domesticus* shares the same set of macro-autosomes, but has a different Z-chromosome and two micro-chromosome pairs which differ by the presence of short arms (see Fig. 33). *G. domesticus* probably shares the addition of a terminal C-band on the p arm of the Z-chromosome with *Coturnix coturnix.* The latter, however, differs by inversions in chromosomes 1, 2 and 4/ m.

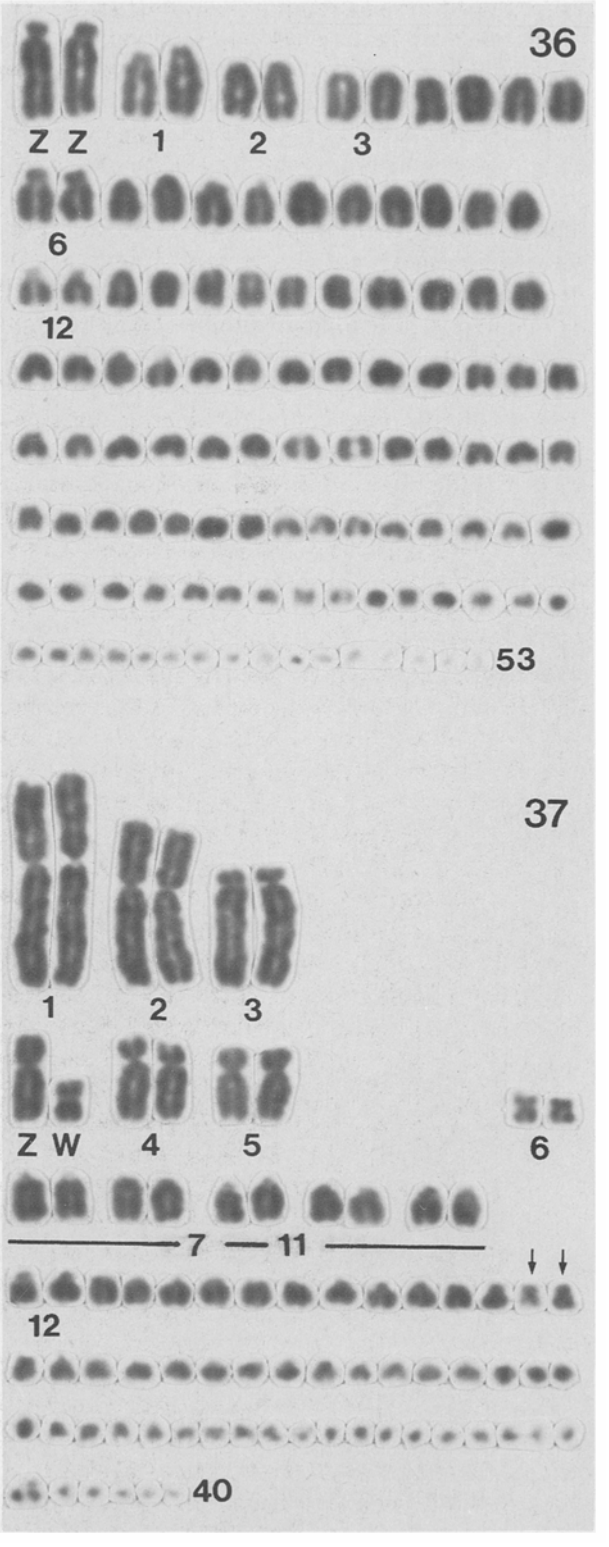
Summarizing, there are four karyological groups of Galliformes, (1) the Megapodiidae, (2) the Cracidae, (3) a group consisting of part of the Phasianidae, the Meleagrididae and Tetraonidae, and (4) a group consisting of the remaining Phasianidae and the Numididae. The Megapodiidae and the non-megapode galliforms are probably sister-groups. The relationship between the three non-megapode groups remains unclear, since no derived characteristics have been detected which are shared by any possible combination of two of them. The same is

true of most of the relationships within each of the above groups 3 and 4. Therefore, the indication of the order in which these groups branched off from the main stem in the phylogenetic tree of the Galliformes presented by Stock & Bunch (1982) does not seem justified. In group 4, for instance, these authors suggested that *Pavo* separated first from the lineage leading to *Gallus,* followed by the Numididae and finally by *Coturnix.* In the absence of data on shared derived karyotypic characteristics, nothing can be said on the sequence of these events, and thus, nothing on intra-group relationships.

One thing, however, is indicated very clearly by the karyological data: the Phasianidae s.s. do not form a natural group (Stock & Bunch, 1982), since a number of them are more closely related to Meleagrididae and Tetraonidae, while the remainder are allied to the Numididae. The question as to what taxonomic status should be given to the various groups of non-megapodes cannot so far be answered by karyological data. The suggestion of Stock and Bunch to lend subfamily rank to each of the above groups 2, 3 and 4 has no karyological basis, since karyotypic differences are not directly related to phylogenetic distances. More precise information on intra- and intergroup relationships may, however, be obtained when more species are studied using the most sophisticated banding techniques.

Gruiformes

Cariama cristata (Cariamidae; Fig. 36), with 2n = 108, has one of the highest diploid numbers as yet recorded in birds. The subtelocentric Z-chromosomes are clearly the largest elements of the karyotype. Whereas the Zs are of the same absolute length as those in many other birds, the largest autosomes, which measure only 70% of the Z, are fairly small. There is a very gradual decrease in size in the autosome series. Except for pairs 6 and 12 which exhibit minute short arms, all autosomes are acrocentric or of uncertain centromeric position.



Figs. 36–37. Representative karyogrammes of Cariamidae and Gruidae: (36) male *Cariama cristata* (magnification 4 000× instead of 3 000× in other illustrations) (compare also Fig. 38); (37) female *Grus japonensis* (small arrows point at minute biarmed microchromosomes).

The W-chromosome could not be distinguished from the autosomes, since no C-banding has been undertaken on material from a female specimen. C-banding on one male individual revealed the presence of an unpaired acrocentric chromosome with a remarkable C-positive proximal segment (Fig. 38). The heterozygous occurrence of this element cannot be explained and there can be no doubt as to this individual's sex since the two Z-chromosomes were clearly recognizable as the largest chromosomes in the complement. De Aguiar (1968) reported a diploid number of 110 for *C. cristata,* but no details on the karyotype are known. Takagi & Sasaki (1980) and Sasaki & Takagi (1981) found a diploid number of 106 in another representative of the family Cariamidae, *Chunga burmeisteri,* a finding since confirmed in our laboratory. Except for the metacentric W-chromosome (which must be acrocentric in *C. cristata*), the karyotype of this species appears to be identical to that described above.

The karyotypes of the Cariamidae differ greatly from those of the Gruidae. Nearly all species of the latter family have been studied cytologically (for references see De Boer, 1984), and except for the possible presence of an extra pair of very small submetacentrics in several species (see Figs. 37 and 39) and of a possibly acrocentric W-chromosome

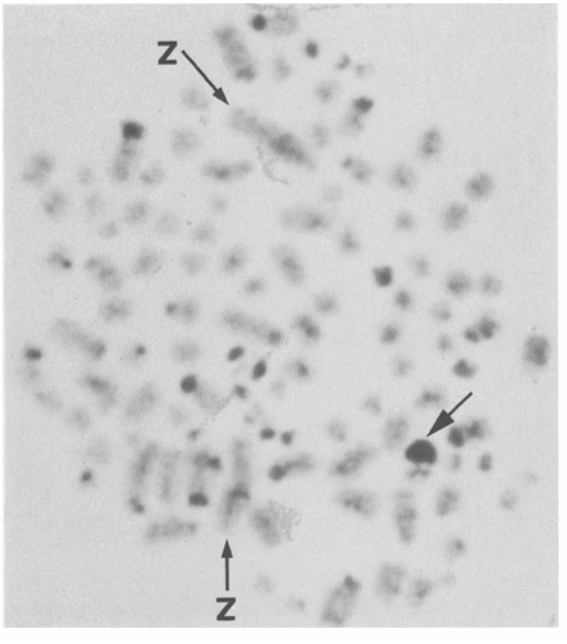
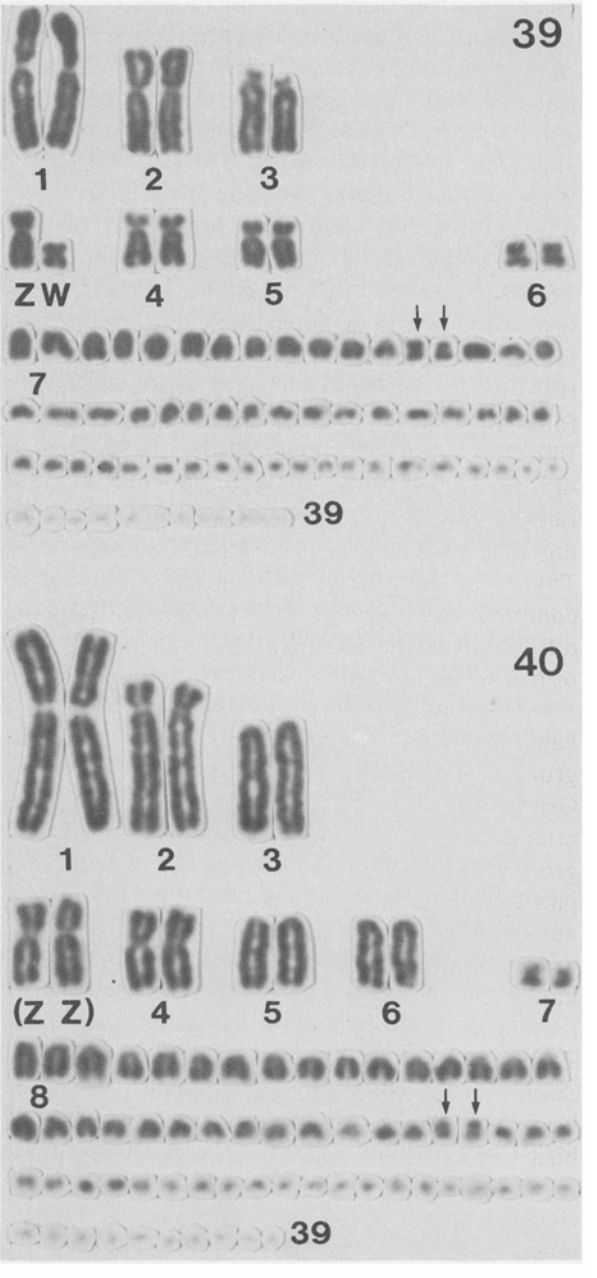


Fig. 38. C-banded metaphase plate of male *Cariama cristata.* Note presence of an unpaired small acrocentric element with heavy C-banding (arrow). This is not a W-chromosome since clearly two Zs are seen.



Figs. 39–40. Representative karyogrammes of Gruidae and Psophiidae: (39) female *Grus rubicunda;* (40) male *Psophia crepitans* (Z-chromosomes identified according to Sasaki & Takagi, 1981). Small arrows point at minute biarmed microchromosomes.

instead of a metacentric one in *Grus antigone* (pers. obs.) all karyotypes are identical. In Figure 37 the karyotype of *Grus japonensis* is shown; Figure 39 shows that of *G. rubicunda* which is presented here for the first time. The standard karyotype of the Gruidae includes 7 pairs of recognizable chromosomes: pairs 1, 2 and 3 are similar in morphology to those seen in various other avian taxa (see discussions on Pelecaniformes and Galliformes), pairs 4 and 5 are medium-sized submetacentrics, pair 6 consists of two small metacentrics, while the Z-chromosomes are submetacentric and of the size of pair 4. All remaining autosomes are acrocentric, gradually decreasing in size from small to minute. The two largest elements of these (pair 7) often are involved in associations with each other. The diploid number is approximately 80. It is very likely that this karyotype is an ancient one. It is not only found in all members of the Gruidae, but also in the Phoenicopteridae (belonging to the Ciconiiformes or forming an order of their own) and in the Cathartidae (belonging to the Falconiformes or constituting an independent order) (De Boer, 1975, 1976; Takagi & Sasaki, 1974). In addition, the most common karyotype of the Columbidae possibly differs only by the absence of the small metacentrics of pair 6 (see the discussion on Columbiformes).

It remains totally unclear how the cariamid karyotype could possibly have evolved from the gruid karyotype which is probably ancestral to the Gruiformes. The largest autosomes of the cariamids are clearly smaller than the short arms of the gruid pairs 1 and 2, while the long arms of the gruid pairs 1, 2 and 3 are twice as long as the longest autosomes. Thus, even fissioning of all biarmed gruid chromosomes would not lead to the cariamid karyotype; further fragmentation of at least the five longest chromosome arms would be necessary. Therefore, it is of great interest that Takagi & Sasaki (1980) discovered clear karyological similarity between the cariama's and a non-gruiform bird: *Ramphastos toco* (Rhamphastidae, Piciformes). However, although the position of the Cariamidae in the Gruiformes has been a matter of much taxonomic debate, a possible alliance of this family with the Rhamphastidae or the Piciformes has never been suggested. Takagi & Sasaki (1980) also noted that the cariamas have almost the same number of chromosome arms as *Sagittarius serpentarius,* sole member of the Sagittariidae, a family whose position in the Falconiformes always has been debated. *Sagittarius* sometimes has been thought to be related to the Cariamidae (for references see Sibley & Ahlquist, 1972) and possesses a karyotype that differs clearly from those of other Falconiformes (which are, by the way, very heterogeneous; De Boer, 1976). Total fissioning of the *Sagittarius* karyotype would indeed result in a karyotype similar to that of *Cariama,* but such a very speculative suggestion would require high-resolution chromosome banding analysis to detect possible homologies in the small chromosome arms of the two species.

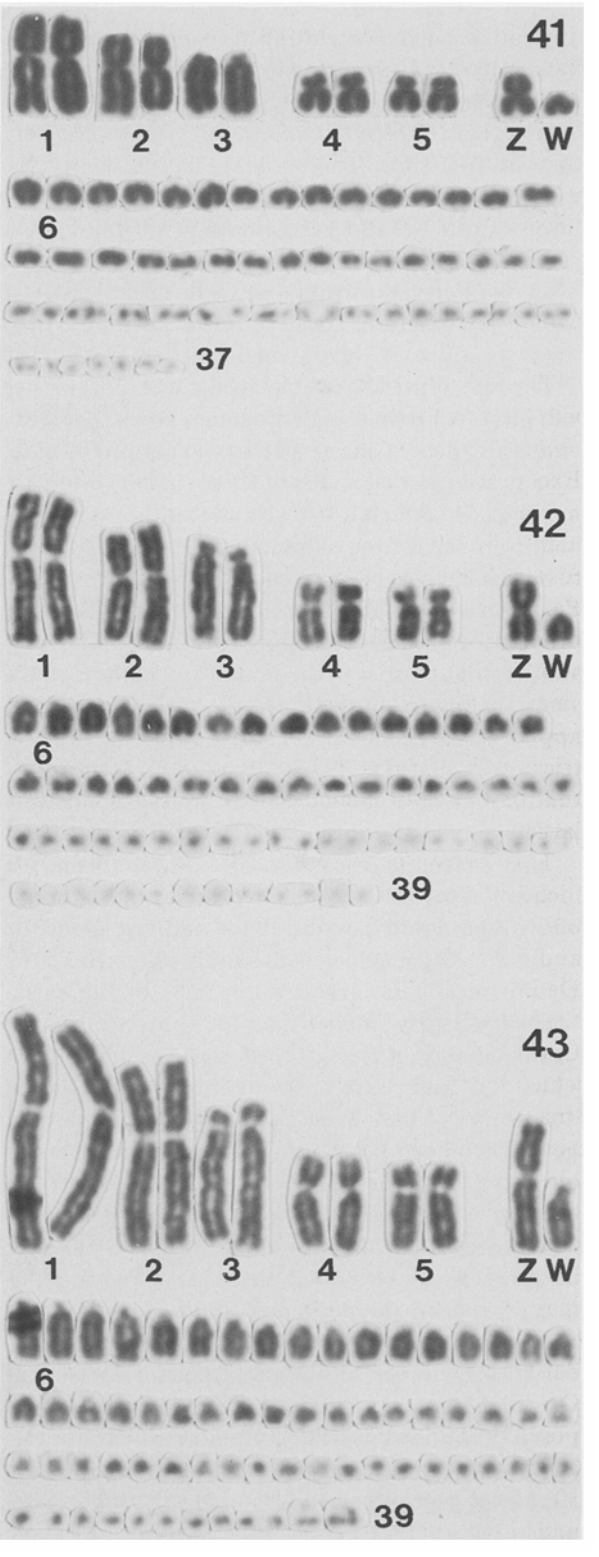
The Psophiidae, also mentioned as possible relatives of the Cariamidae, karyologically are much closer to the Gruidae than the cariamas. Figure 40 shows the karyotype of *Psophia crepitans* which is identical to those of *P. leucoptera* and *P. viridis* (Sasaki & Takagi, 1981). *Psophia* differs from the cranes by the higher arm-ratio of pair 2 (5.3 instead of 1.6, possibly the result of pericentric inversion), the absence of the minute short arms in pair 3 (possibly brought about by pericentric inversion or translocation), the lower arm ratio of pair 4 (1.5 instead of 2.7, possibly resulting from pericentric inversion), the acrocentric morphology of pair 5 (possibly by pericentric inversion) and the presence of an additional pair of medium-sized chromosomes (pair 6), which may have arisen from tandem fusion of relatively large microchromosomes. As in the cranes there is a pair of small metacentrics (pair 7), the remaining microchromosomes are acrocentric with the possible exception of two small elements; see Fig. 40) and the diploid number is approximately 80. The suggested homologies and the rearrangements that may have given rise to the differences between Gruidae and Psophiidae need to be confirmed by banding studies.

Of the remaining gruiform families that have been studied cytologically to date, the Otididae karyologically are closest to the Gruidae. Detailed banding studies by Nishida *et al.* (1981) showed that *Otis tarda* differs from the gruids only by a small pericentric inversion in pair 5 and a pericentric inversion in the Z-chromosome. If our above suggestion that the karyotype of the Gruidae is original to the order is right, the retention of most of the original characteristics in the Otididae, however, would not prove close relationship between these two families.

Three species of Rallidae studied cytologically, *Fulica atra, Gallinula chloropus* and *Porphyrio poliocephalus* (Hammar, 1970; Itoh *et al.*, 1969) are characterized by the presence of a submetacentric pair 3, possibly derived from the subtelocentric pair 3 of the Gruidae by pericentric inversion. The former two species exhibit metacentric pairs 4 and 5 (submetacentric in the Gruidae), possibly also derived by pericentric inversions, and sex chromosomes similar to those of the gruids. The latter species, of which no definite female was studied (Itoh *et al.*, 1969), possesses only two pairs of medium-sized biarmed (metacentric) chromosomes. Since one of these is likely to be the Z-chromosome pair, either pair 4 or pair 5 of the former two rallids seems to be absent, possibly due to fissioning. In a fourth species of Rallidae, *Amaurornis phoenicurus* studied by Misra (1974), pair 3 appears to have retained the original subtelocentric morphology as in the Gruidae. Like *Porphyrio poliocephalus,* this species lacks one of the biarmed autosome pairs 4 or 5. It is unclear whether or not the four rallids mentioned above possess the characteristic pair of small metacentrics found in Gruidae (pair 6) and Psophiidae (pair 7). Detailed information on the karyotypic morphology of several other rallids studied to date (for references see De Boer, 1984) is lacking.

The karyotype of *Rhynochetos jubatus,* sole representative of the family Rhynochetidae, differs considerably from those of the Gruidae, Psophiidae and Rallidae. Although the karyotypes presented by Hoffmann *et al.* (1974) are not of high quality, it is clear that this species at least lacks pairs 1, 2, 4 and 5 of the gruid karyotype, all of which may have fissioned. More detailed comparison, however, would require better material of *R. jubatus.*

None of the remaining gruiform families, Mesitornithidae, Turnicidae, Pedionomidae, Aramidae, Heliornithidae and Eurypygidae have been studied cytologically so far. Of the six families treated above, only the Rallidae show clear karyological variation. The Gruidae, Psophiidae and Cariamidae do not seem to exhibit intra-familiar variation, while of Rhynochaetidae and Otididae only one species each was studied. On the other hand, no two of these six families are karyologically identical. For the time being, however, nothing can be said on the possible inter-familiar karyological relationships. The characteristics by which Cariamidae, Psophiidae, Rallidae, Rhynochaetidae and Otidi-



Figs. 41–43. Representative karyogrammes of Columbidae: (41) female *Goura cristata;* (42) female *G. scheepmakeri;* (43) female *Caloenas nicobarica.*

dae differ from the supposed ancestral Gruidae karyotype may have originated independently. Therefore, it would be of great interest to study more species of the poorly documented families and to collect data on the hitherto unstudied groups. It is possible this could shed some more light on the uncertain relationships within the order.

Columbiformes

Three new karyotypes of Columbidae were studied, those of *Goura cristata* (Fig. 41), *G. scheepmakeri* (Fig. 42) and *Caloenas nicobarica* (Fig. 43). Except for the clearly greater length of the Z-chromosome in *C. nicobarica,* all three karyotypes are identical. They show five recognizable pairs of autosomes: pairs 1, 2 and 3 are similar to those of Pelecanidae, Gruidae, Phoenicopteridae and many other groups, while pairs 4 and 5 are medium-sized submetacentrics. The remaining autosomes are small to minute acrocentrics; the diploid number is approximately 80. The Z of *Goura* spp. is metacentric and slightly larger than the autosomes of pair 4, that of *Caloenas* is metacentric as well, but much larger.

This karyotype is very similar or possibly even identical to that of *Goura victoria, Zenaida* (=*Zenaidura*) *macroura, Columba livia, C. palumbus* and several other columbids (for references see De Boer, 1984). In addition, it very much resembles the karyotypes of Gruidae, Phoenicopteridae and Cathartidae (see discussion of Gruiformes), from which it differs mainly by the absence of a pair of small metacentrics. It would be valuable to clearly assess the possible homologies between the columbid karyotype and those of the other groups mentioned by detailed banding analyses, since we may be dealing with a rather old karyotype. Because of its presence in various genera of Columbidae, it may in any case be ancestral to this family.

This, however, does not mean that the Columbidae would be a karyologically homogeneous group. On the contrary, they show quite some variation, possibly brought about by fissions, fusions, pericentric and paracentric inversions. To date, over 30 species have been studied (see De Boer, 1984) and discussions on karyotypic evolution in the family have been presented by De Lucca & De Aguiar (1976, 1978) and De Lucca (1984). Nevertheless, our picture of the karyology of Columbidae seems to be rather incomplete. Further extensive investigations in this group would be highly interesting.

Cuculiformes

Our results of *Musophaga violacea* (Fig. 44) are rather poor. Nevertheless we present them because hitherto only a single species of the Musophagidae has been studied, viz. *Tauraco* (=*Gallirex*) *porphyriolophus* (Stock & Mengden, 1975). *M. violacea* has a diploid chromosome number of approximately 82. It possesses no really large autosomes. Pair 1 is acrocentric, pairs 2–4 are subtelocentric, pair 5 is submetacentric. The remaining autosomes are probably acrocentrics of gradually decreasing size. The Z-chromosome is a submetacentric of the size of pair 3. The W-chromosome of the female specimen studied could not be identified. As far as can be discerned, this karyotype is identical to that of *T. porphyriolophus* (Stock & Mengden, 1975).

Several species of the second cuculiform family, the Cuculidae, have been studied cytologically (for references see De Boer, 1984). However, detailed information on their karyotypic structure is currently unavailable. Nevertheless, comparison of the musophagid karyotypes with, for instance, the picture of *Guira guira* published by De Lucca (1974b) shows that Musophagidae and Cuculidae are fairly different. Further studies are required to assess the exact karyological relationships between these two cuculiform families.

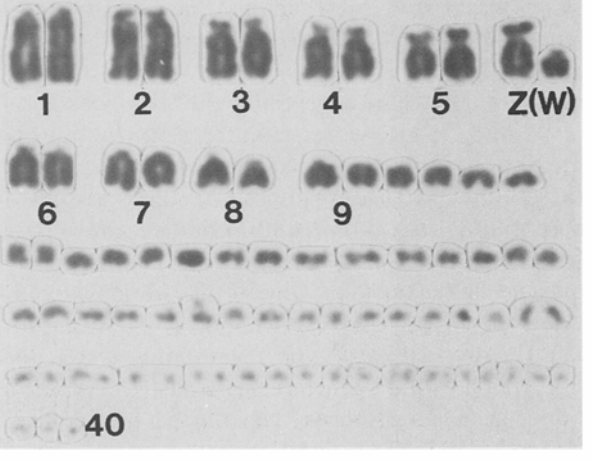


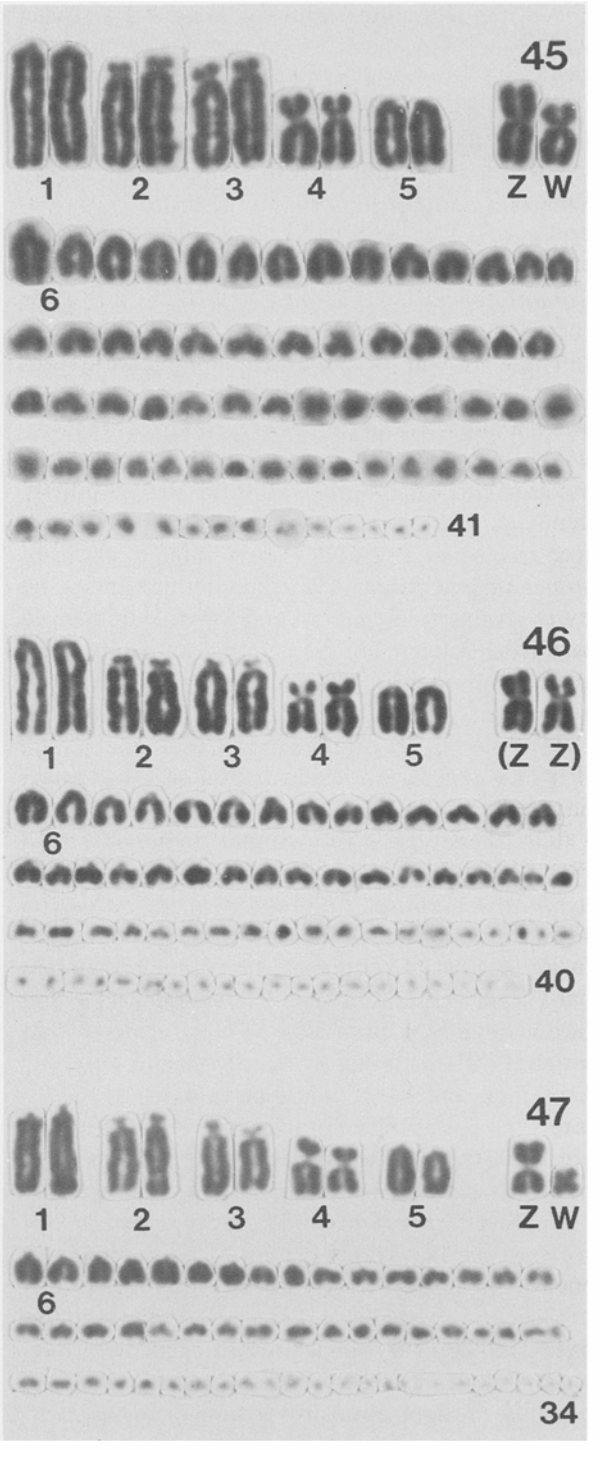
Fig. 44. Representative karyogramme of female *Musophaga violocea* (Musophagidae).

Strigiformes

Seven species of the family Strigidae were studied, five of which are new to cytology, viz. *Ketupa zeylonensis, Otus leucotis, Bubo africanus, Ciccaba woodfordii* and *Ninox novaeseelandiae*. Of the remaining two, *Nyctea scandiaca* was studied previously by Sasaki *et al.* (1981) who did not illustrate the karyotype, while that of *Bubo bubo* was published by De Boer (1976).

The karyotypes of *Nyctea scandiaca, Ketupa zeylonensis* and *Otus leucotis* (Figs. 45–47) are identical and show five pairs of recognizable macro-autosomes. Pair 1 consists of large acrocentrics. Pairs 2 and 3 are subtelocentrics of almost the same size, but slightly smaller than pair 1. Pair 4 consists of medium-sized submetacentrics, while the elements of pair 5 are somewhat smaller acrocentrics. There are approximately 70 small to minute acrocentric autosomes (2n = 82), the largest of which measures approximately two-thirds of the length of pair 5. The Z-chromosome is metacentric, slightly larger than the submetacentrics of pair 4. The W is metacentric in *O. leucotis,* submetacentric and somewhat larger in *N. scandiaca.* No female of *K. zeylonensis* was studied.

The same karyotype was reported to occur in *Bubo virginianus* (Biederman *et al.,* 1980; Krishan *et al.,* 1965), *Ketupa ketupa* and *K. blakistoni* (Sasaki *et al.,* 1981). The same morphology of the five pairs of macro-autosomes was found in *Asio otus* and *flammeus* (Sasaki *et al.,* 1981; Biederman, unpublished, see Shields, 1982), *Otus scops* (Itoh *et al.,* 1969), *O. asio* (Sasaki *et al.,* 1981) and *Glaucidium radiatum* (Misra, 1974). Because of the presence of these five pairs in a variety of genera, their morphology may be ancestral to the Strigidae. *Otus scops* and *O. asio* differ from *O. leucotis* by the presence of three additional pairs of medium-sized to small biarmed autosomes. As these are also absent in *Nyctea ketupa* and *Bubo* (see above), as well as in *Strix* and *Ciccaba* (see below), their presence in the two *Otus* species may be considered a derived characteristic. *O. leucotis* would then present the original karyotype of the genus, a conclusion which would be in agreement with the suggestion that it is a primitive, generalized species (see Hekstra, 1973). Two additional pairs of medium-sized to small biarmed autosomes were found in *Asio otus* and *A. flammeus* (Sasaki *et al.,* 1981;



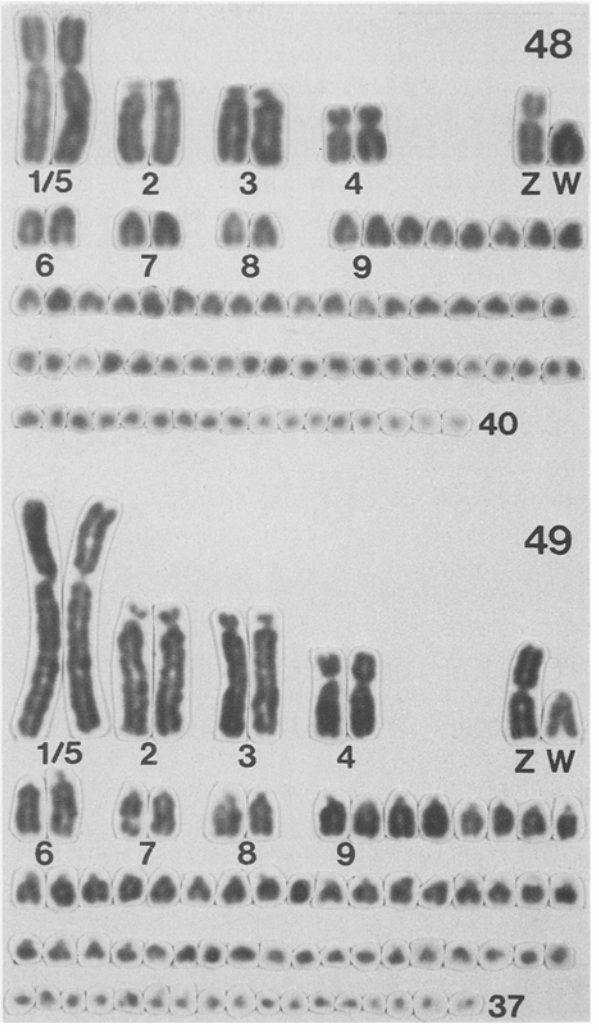
Figs. 45–47. Representative karyogrammes of Strigidae: (45) female *Nyctea scandiaca;* (46) male *Ketupa zeylonensis* (Z-chromosomes tentatively chosen on the basis of comparison with other strigids); (47) female *Otus leucotis.*

Biederman, unpublished, see Shields 1982). A single additional pair of such elements was reported to occur in *Glaucidium radiatum* (Misra, 1974; Misra & Srivastava, 1974). It remains uncertain whether the additional metacentrics of *Asio* and *Glaucidium* evolved partly in common or from independent fusions of microchromosomes. Those of *Otus* (*asio* and *scops*) must have evolved independently, since they are not found in *O. leucotis* (see Fig. 52).

The remaining representatives of the Strigidae that have been studied cytologically, can be arranged in four groups, each characterized by a specific morphology of the five pairs of macro-autosomes. The first group includes *Bubo bubo* and *B. africanus* (Figs. 48 and 49), which differ from the supposed ancestral karyotype by the fusion of acrocentric pairs 1 and 5 (pair '1/5'. *B. bubo* differs from *B. africanus* by its submetacentric Z-chromosome. The metacentric Z of *B. africanus* is shared by *B. virginianus* (see above). Both *B. bubo* and *B. africanus* differ from *B. virginianus* by their acrocentric W, which is metacentric in the latter species as well as in most other strigids.

The second group comprises *Ciccaba woodfordii* (Fig. 50) and *Strix,* of which to date four species have been studied, viz. *S. aluco* (Renzoni & Vegni-Talluri, 1966; Hammar, 1970), *S. leptogrammica* (Sasaki *et al.,* 1981), *S. nebulosa* (Biederman, cited in Shields, 1982) and *S. uralensis* (Takagi & Sasaki, 1974). These two genera are characterized by the fusion between the original pair 5 and one of the pairs of small acrocentrics, possibly pair 7, resulting in a pair of submetacentrics (pair '5/7' almost as large as pair 3. *Ciccaba woodfordii* differs from *Strix* spp. and probably most other owls by the larger Z-chromosome, possibly caused by an increase in length of the long arm. The metacentric W of this species is also relatively large.

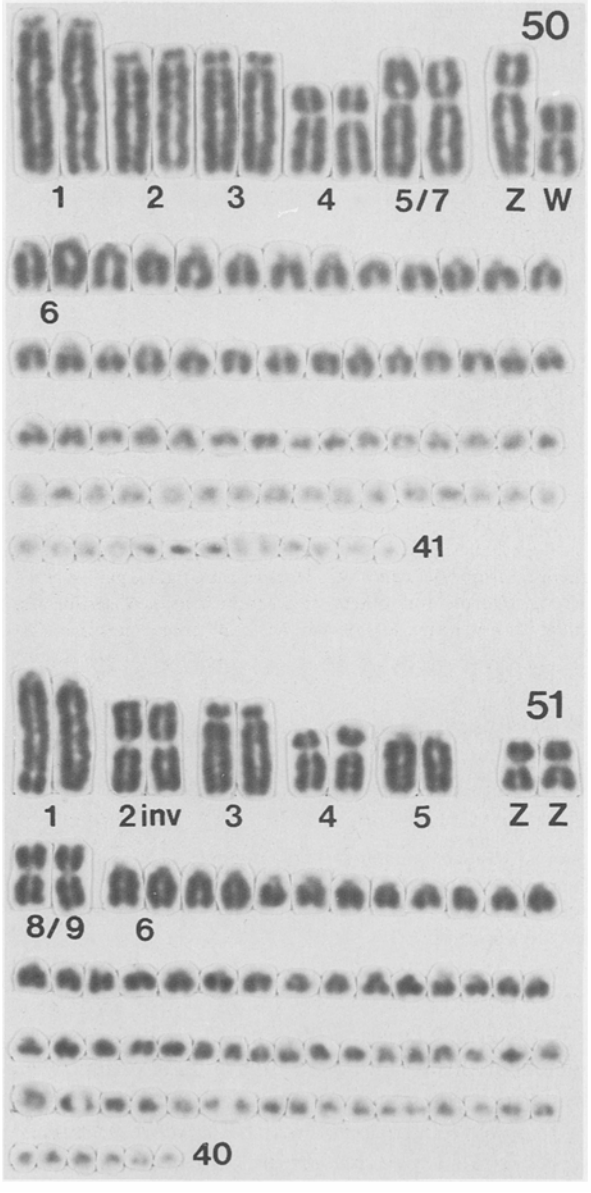
The third group, including only *Ninox novaeseelandiae* (Fig. 51), is characterized by the presence of a pair of large metacentrics of the size of the original subtelocentrics of pairs 2 and 3. Since one of these pairs is absent the large metacentrics may have evolved by pericentric inversion of pair 2 or 3 (in Fig. 51 they are provisionally indicated as pair '2inv'). Apart from this change in the macro-autosomes, *N. novaeseelandiae* shows an additional pair of medium-sized metacentrics (slightly smaller than pair 5) which possibly evolved from fusion between the original small acrocentrics of pairs 8 and 9 (pair '8/9'). The Z-chromosome is metacentric and similar to that of most other owls; the W is metacentric as well.



Figs. 48–49. Representative karyogrammes of Strigidae: (48) female *Bubo bubo;* (49) female *B. africanus.* Pair 1/5 supposedly evolved from fusion between pairs 1 and 5 of the ancestral strigid karyotype (see Fig. 52).

The fourth group, of which no representatives were studied by the present authors, includes *Athene brama* (Ray-Chaudhuri *et al.,* 1969; Misra, 1974; Misra & Srivastava, 1974), *A. noctua* (Renzoni & Vegni-Talluri, 1966) and *Pulsatrix perspicillata* (Takagi & Sasaki, 1974). They are characterized by the presence of a very large pair of metacentrics and the absence of the original sub-

telocentric pairs 2 and 3. Thus these pairs probably fused to form pair '2/3' (Fig. 52). In addition both species of *Athene* lack the acrocentric pair 5 which is replaced by a pair of metacentrics of the same size, a change probably caused by pericentric inversion (indicated by '5inv' in Fig. 52). They also show



Figs. 50–51. Representative karyogrammes of Strigidae: (50) female *Ciccaba woodfordii* (pair 5/7 supposedly evolved from fusion between pairs 5 and 7 of the ancestral strigid karyotype); (51) male *Ninox novaeseelandiae* (pair 8/9 supposedly evolved from fusion of pairs 8 and 9 of the original strigid karyotype, pair 2inv from pericentric inversion in pair 2).

the presence of an extra pair of medium-sized metacentrics which may have evolved from the fusion of small acrocentrics. Since Takagi & Sasaki (1974) did not publish an illustration of the karyotype of *P. perspicillata,* it is not clear whether this species also shares pair 5inv and the additional medium-sized metacentrics with *Athene.*

All of the changes in the five supposed original pairs of macro-autosomes by which the above four groups of owls are characterized, apparently occurred independently and most probably express relationships within each group (*B. bubo-B. africanus, Strix-Ciccaba, Athene-Pulsatrix,* while *Ninox* stands alone). It is possible that detailed banding studies may reveal conclusive evidence as to the question of whether or not the additional pairs of medium-sized to small biarmed autosomes in *Ninox,* the *Athene-Pulsatrix* group and in *Glaucidium* and *Asio* (see above) evolved (partly) in common. So far, nothing can be said with certainty on this matter. In Figure 52 a summary is given of the supposed macrochromosomal evolution in the Strigidae.

The karyotype of *Tyto alba* was found by Renzoni & Vegni-Talluri [(1966), whose karyotype for the species was republished by Ray-Chaudhuri (1973)] to differ greatly from those of the strigids. The species has a relatively high diploid number (approximately 92) in comparison to the strigids. In addition it lacks large autosomes. The Z-chromosome, probably of the same size as that of the Strigidae, clearly is the largest element in the set. It is the only metacentric chromosome and thus easily recognizable; all autosomes seem to be acrocentric. We restudied the species and although our results are of the same poor quality as those of Renzoni & Vegni-Talluri (1966) a metaphase plate is shown in Figure 53, because our findings disagree with theirs with respect to the morphology of the W-chromosome. Renzoni and Vegni-Talluri reported the W to be a large metacentric element, only slightly smaller than the Z. In our two females no such chromosome was present; the W must be one of the small acrocentrics. For the remainder we confirmed the previous findings.

The karyotype of *Phodilus badius* (Fig. 54) bears clear resemblance to that of *Tyto alba.* It also has a diploid number of approximately 92, also lacks any large chromosomes and includes one pair of medium-sized metacentrics which are clearly larger than

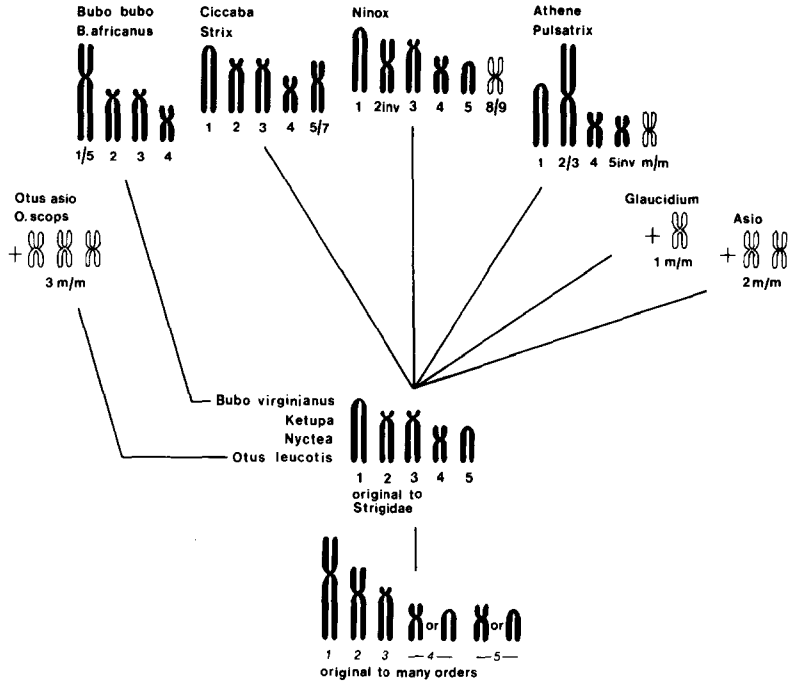
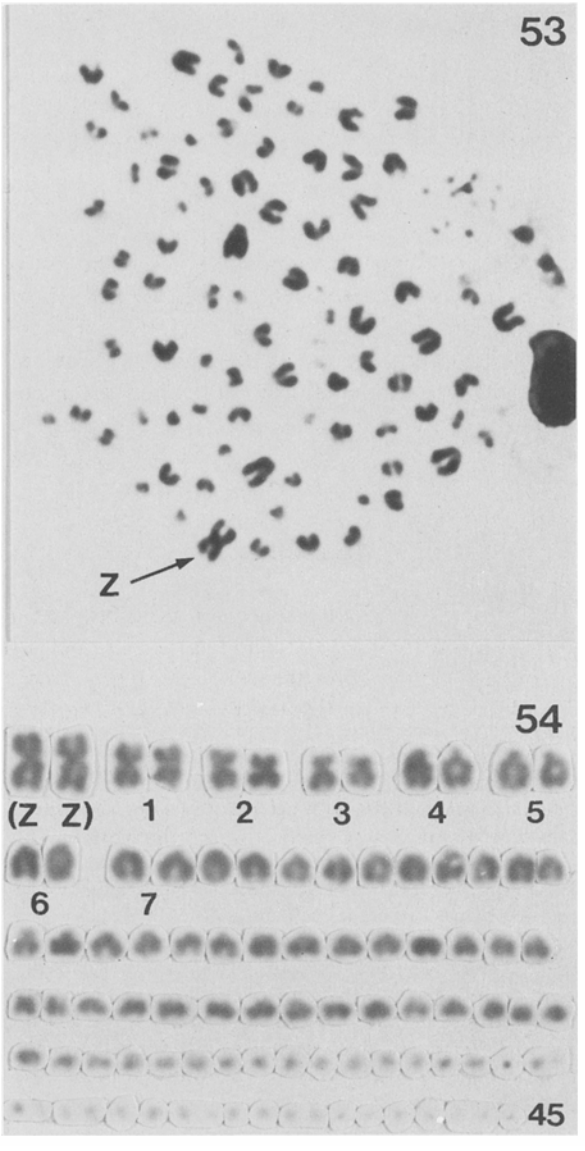


Fig. 52. Schematic representation of the possible evolutionary changes in the macro-autosomes of the Strigidae. The ancestral strigid karyotype with five pairs of macro-autosomes supposedly evolved from a karyotype ancestral to various bird orders by fissioning of pair *1* (giving rise to strigid pairs 2 and 5), pericentric inversion in pair *2* (giving rise to strigid pair 1), retention of subtelocentric pair *3* and submetacentric pair *4* (strigid pairs 3 and 4) and loss of pair *5* as a macrochromosome (it is uncertain whether the original pairs *4* and *5* were subtelocentric as in Gruiformes, Pelecaniformes, Ciconiiformes, Columbiformes and others, or acrocentric as in Anseriformes, Galliformes and Ratitae). The supposed original strigid karyotype is characterized by the loss of pair *5* as a macrochromosome. The supposed original strigid karyotype is still found in *Bubo virginianus, Otus leucotis, Ketupa* and *Nyctea* (in the scheme generic names are used when so far only a single karyotype has been found in a genus; when different karyotypes exist, species names are used). The chromosome nomenclature used in this scheme is also used in the illustrated strigid karyotypes of Figures 45–51. 1/5 indicates a chromosome 1 fused with chromosome 5, 5/7 a chromosome 5 fused with chromosome 7, 8/9 the product of a fusion between two microchromosomes (probably numbers 8 and 9), 2/3 the fused chromosomes 2 and 3, 2inv and 5inv pericentrically inverted chromosomes 2 and 5. Unidentified products of fusions between microchromosomes are indicated with 'm/m'. All elements that evolved from fusions between microchromosomes are given in outline; the original macrochromosomes in black. As no information is available on possible homologies between pair 8/9 in *Ninox* and the m/m pairs of *Pulsatrix, Glaucidium* and *Asio* it remains unclear whether or not these pairs could have evolved (partly) in common. The m/m pairs of *Otus asio* and *O. scops* probably evolved independently from those in the other species since the congeneric *O. leucotis* possesses the original karyotype.

the remaining elements. We could study only a male specimen, but believe that in analogy with *Tyto* these metacentrics could represent the Z-chromosome pair. *Phodilus* differs from *Tyto* in the presence of three pairs of metacentric to submetacentric autosomes. These may be related to acrocentrics of *Tyto* through pericentric inversions.

The taxonomic position of *Phodilus* has been matter of some debate. Some authors include the genus with *Tyto* in the Tytonidae, others consider it as intermediate between Tytonidae and Strigidae, include it in the Strigidae or believe it to be different from both (for a review of classification see Sibley & Ahlquist, 1972). Karyologically *Tyto* and *Phodilus* are undoubtedly much more similar to each other than to any member of the Strigidae. This, however, only expresses close relationship between *Tyto* and *Phodilus* if their karyotypic structure does not have to be regarded as original to all Strigiformes. In this respect it can be said that karyotypes closely resembling that of *Tyto* and *Phodilus* are unknown in non-strigiform birds. The possible similarity to the karyotypes of Cariamidae (Gruiformes) and Rhamphastidae (Cuculiformes),



Figs. 53–54. Chromosomes of the Tytonidae: (53) metaphase plate of female *Tyto alba;* (54) karyogramme of male *Phodilus badius* (Z-chromosome pair tentatively chosen on the basis of comparison with *T. alba*). Magnification in both illustrations is the same as that in the strigid owls of Figures 45–51.

which share the absence of large autosomes and the presence of a Z-chromosome that is the largest element, is only superficial since the diploid numbers in these groups are much higher (approx. 110). On the other hand, the supposed original karyotype of the Strigidae (see above) can be easily derived from the karyotypes of many other birds. If the macro-autosomes are compared to the largest pairs (especially pairs 1–3) of Pelecanidae, Ciconiiformes, Anseriformes, Gruidae, Cathartidae, Galliformes and Columbiformes, the following possible homologies are observed. The original strigid acrocentric pair 1 has the same length as submetacentric pair 2 of the above groups and could have originated by pericentric inversion. The strigid pair 2 is of the same length as the long arm of pair 1, while the strigid pair 5 has the same length as the short arm of this pair; thus, the original pair 1 may have fissioned in the ancestral Strigidae. The strigid pair 3 is identical to pair 3 in the other groups. Pair 4 of the Strigidae may correspond to one of the two pairs of medium-sized autosomes as found in several of the above taxa; the second pair of this size found in these taxa is absent in the supposed ancestral strigid karyotype. The diploid number of the Strigidae and the absence of biarmed elements among the small to minute chromosomes is shared by many other birds.

Preliminary banding studies on *Bubo virginianus* and *Pulsatrix perspicillata* by Biederman *et al.* (1980) and Takagi & Sasaki (1974) seem to support the suggested homologies, although more detailed analyses are needed to confirm them. Nevertheless it seems much more likely that the strigid karyotypes evolved directly from a generalized avian karyotype, than from that of *Tyto*. Thus, the karyological similarity between *Tyto* and *Phodilus* most probably indicates true relationship. How their karyotypes evolved from the ancestral strigiform karyotype remains unclear. It also remains obscure to which particular other order the Strigiformes are most closely related karyologically, since many groups show similar macro-chromosomes or differ from the common type only by one or a few rearrangements. Due to the many rearrangements that are found to have occurred within the Strigidae, this family is an ideal subject for further karyotaxonomical studies.

Caprimulgiformes

The Caprimulgiformes belong to the avian orders which have been least studied cytologically. In the literature references are found to three members of the Caprimulgidae (Bulatova *et al.*, 1971; Kaul & Ansari, 1983; De Lucca & Waldrigues, 1983), but to

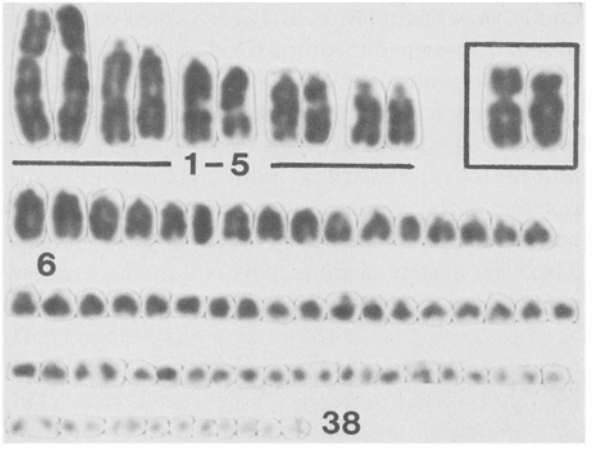


Fig. 55. Representative karyogramme of a specimen of *Podargus strigoides* (Podargidae) of unknown sex. Sex chromosomes in a proven female could not be identified. The inset shows the heteromorphic pair 1 of another specimen of unknown sex.

date detailed information on karyotypic structure is unavailable for them. The present authors studied the chromosomes of *Podargus strigoides*, belonging to the Podargidae. In the absence of data on other Caprimulgiformes the results are presented below without discussion.

The diploid number of *Podargus strigoides* (Fig. 55) is approximately 72. Only the two longest chromosomes are submetacentric, all others are acrocentric. Pair 2 is recognizable because of its large size. Pair 3, 4, and 5 are of medium size. From pair 6 (measuring 2/3 the length of pair 5) on, chromosome length decreases gradually from small to minute. Although one of the three specimens studied was a female (she once laid an egg), no heteromorphic sex-chromosome pair could be found. Thus, both Z and W may belong to the small to minute acrocentrics that cannot be identified individually. C-banding was not undertaken on the material of this species.

Clear heteromorphism was found in pair 1 of one of the other two specimens: one of the elements of this pair had the same arm ratio as that in the other animals (2.1), the other was more subtelocentric (arm ratio 3.5). Since the total length of both homologues is the same, we may be dealing with a pericentric inversion which occurs as a polymorphism or as an individual aberrancy.

Coraciiformes

Five species of the Bucerotidae were studied, a family on which no cytological data were available to date. Four different karyotypes were found. *Aceros undulatus* (Fig. 56) has a diploid number of approximately 70 and its karyotype lacks any large macrochromosomes: the subtelocentric Z-chromosome is the largest element of the set. The autosomes of pair 1, subtelocentrics with minute short arms, are almost as large as the Z. Pairs 2 and 3 are smaller subtelocentrics, while pair 4 consists of metacentrics. All remaining autosomes are acrocentrics, gradually decreasing from medium to minute size. Only between pairs 15 and 16 is there a noticeable drop in size. There are relatively few really small microchromosomes. C-banding revealed that the W is a small acrocentric element of the size of pair 18.

Buceros bicornis, like *Aceros undulatus,* has a diploid number of 70 and lacks large macrochromosomes, while there are very few really small microchromosomes. However, among its largest 13 pairs there are many more elements with distinct short arms (Fig. 57). In fact only a single pair in this group is clearly acrocentric (number 12). The metacentrics of pair 4, clearly distinguishable in *A. undulatus,* are absent in *B. bicornis.* The Z of the latter species has a slightly higher arm ratio (7.0)

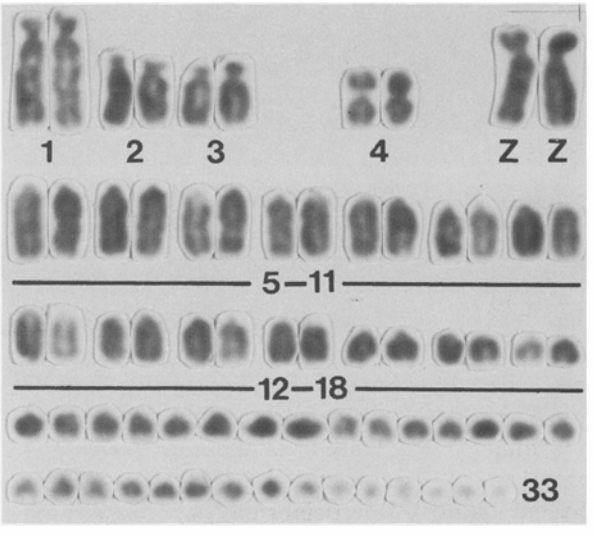
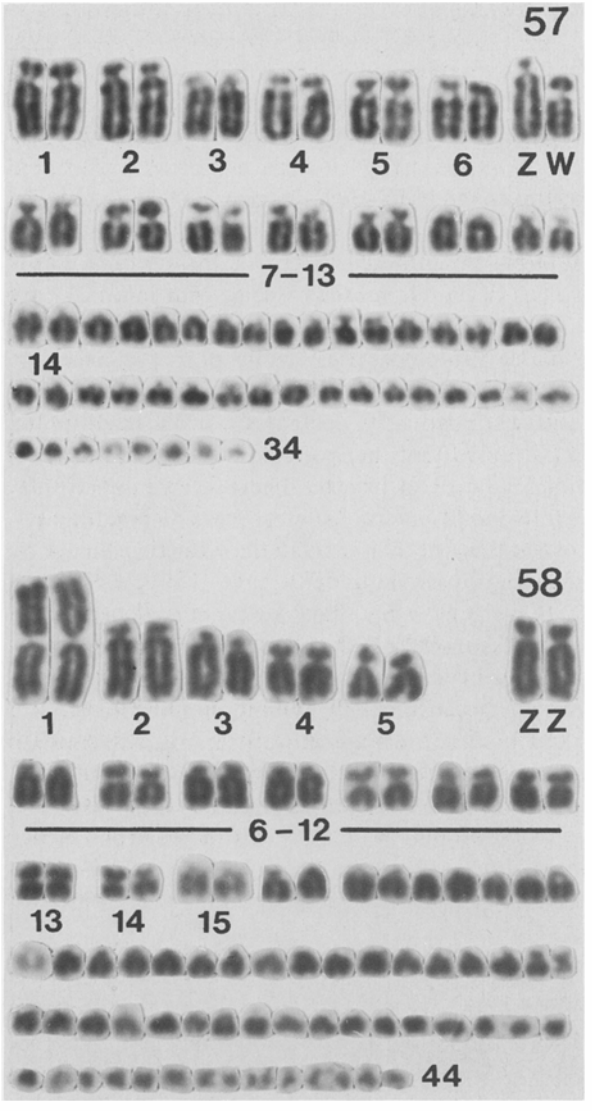


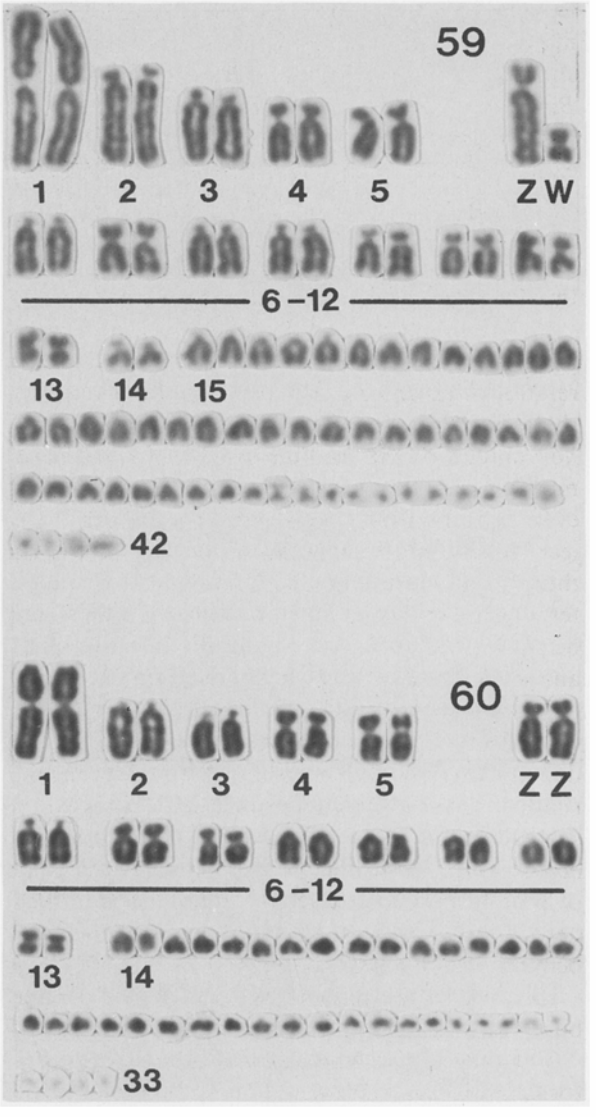
Fig. 56. Representative karyogramme of male *Aceros undulatus* (Bucerotidae).



Figs. 57–58. Representative karyogrammes of Bucerotidae: (57) female *Buceros bicornis;* (58) male *Bucorvus abyssinicus.*

than that of the former (4.8). The W is a submetacentric element.

The karyotypes of *Bucorvus abyssinicus* (Fig. 58) and *B. leadbeateri* (Fig. 59) probably are identical, but differ considerably from those of *Buceros bicornis* and *Aceros undulatus.* With 2n = ±88 their diploid number is much higher, an observation made in many good metaphase plates. In addition, they show the presence of one pair of large metacentric macrochromosomes not found in *Buceros* and *Aceros* (pair 1). As in *Buceros bicornis,* there are many biarmed medium-sized to small chromosomes (among pairs 2–14 only the numbers 6, 8 and 9 are clearly acrocentric), but even in this group there seem to be several differences with regard to the morphology of individual pairs. The remaining autosomes of the two *Bucorvus* species are acrocentric and gradually decrease in size from small to minute. There are clearly more really small microchromosomes than in *Buceros* and *Aceros,* so that the



Figs. 59–60. Representative karyogrammes of Bucerotidae: (59) female *Bucorvus leadbeateri;* (60) male *Tockus fasciatus* (Z-chromosomes tentatively chosen on the basis of comparison with other bucerotids).

higher diploid number seems to result (mainly) from the increased number of these elements. The Z-chromosome is subtelocentric as in the above species (A.R. 4.5). It is of the size of pair 2. The W probably is a small submetacentric chromosome.

Tockus fasciatus (Fig. 60) shares the relatively low diploid number (2n = ±68) with *Aceros* and *Buceros,* but on the other hand it has a single pair of large metacentric macrochromosomes (pair 1) in common with *Bucorvus.* Like *Buceros* and *Bucorvus, T. fasciatus* has relatively many medium-sized to small biarmed autosomes (pairs 2–8 and 12). The morphology of these elements seems to resemble slightly more closely that of *Buceros* than that of *Bucorvus.* However, in the absence of detailed banding patterns it is extremely difficult to trace exact homologies in this group of chromosomes. Although only a male specimen was studied, a pair of subtelocentrics was tentatively chosen as representing the Z-chromosome pair in accordance with the Zs of the other bucerotids.

At this moment it would be premature to speculate on the possible chromosomal evolution and relationships among the Bucerotidae, because of the lack of conclusive evidence as to interspecific homologies of the medium-sized and small chromosomes in their karyotypes. It is evident, however, that this group constitutes an attractive subject for further karyotaxonomic studies, because of the great karyological divergence exhibited by the few species studied so far. If, for instance, it could be proved that a karyotype like that of *Aceros* was ancestral to the family (low 2n, no large metacentric pair and no medium-sized to small biarmed autosomes) clear taxonomic conclusions could be drawn. *Buceros, Bucorvus* and *Tockus* would then share a relatively high number of biarmed autosomes as a derived characteristic, *Tockus* and *Bucorvus* would share the derived large metacentrics of pair 1, and *Bucorvus abyssinicus* and *B. leadbeateri* would share the derived high diploid number. At this moment, however, there is insufficient evidence as to possible composition of the ancestral burecotid karyotype.

The karyological knowledge of the other Coraciiform families is extremely limited. Data on Todidae, Momotidae, Brachypteraciidae, Leptosomatidae and Phoeniculidae are completely lacking. A single species of the Meropidae has been studied by Srivastava & Misra (1973) *(Merops orientalis)* but no information on karyotypic morphology was given. Misra & Srivastava (1975) described the karyotype of *Upupa epops* (Upupidae), which has a rather aberrant chromosome set. It has the highest diploid number found in birds (approx. 120) and shows only one or two pairs of autosomes that measure more than half the length of the Z-chromosomes. All others are small to very small. Karyotypes with a similar general morphology are only found in *Cariama cristata* and *Rhamphastos toco* (see discussion on Cariamidae), but this similarity is most probably superficial.

The karyotypes of two species of *Coracias* (Coraciidae) are known, *C. garrulus* (Bulatova, 1977) and *C. benghalensis* (Misra, 1974; Ray-Chaudhuri, 1976). The latter species was also studied by the present authors and its karyotype is shown for comparison in Figure 61. As in the case of *C. garrulus,* this species has a diploid number of approximately 88 and shows two pairs of large macrochromosomes (pair 1 submetacentric, pair 2 subtelocentric). Apart from four pairs of small biarmed autosomes (pair 3–6) all remaining autosomes are acrocentric as far as their centromeric positions are identifiable. Among these elements there is a gradual decrease in length from small to minute. The Z-chromosome is medium-sized (clearly smaller

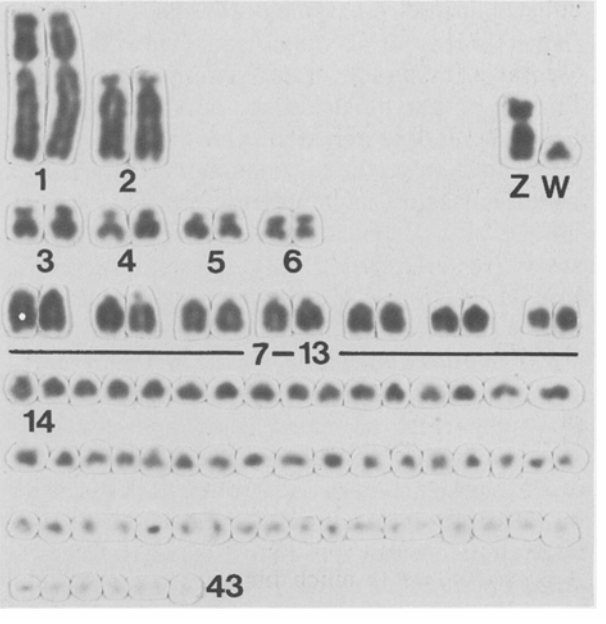


Fig. 61. Representative karyogramme of female *Coracias benghalensis* (Coraciidae).

than the autosomes of pair 2) and submetacentric. The W is small and probably submetacentric. The karyotypic morphology of *Coracias* is clearly different from those of the above five species of Bucerotidae. Due to the lack of evidence on chromosome homologies no speculations are justified on possible relationships between both families.

The same is true for the last Coraciiform family for which karyological information is available, the Alcedinidae. Two members of this family were studied, *Halcyon smyrnensis* (Kaul & Ansari, 1983) and *Dacelo novaeguineae* (=*D. gigas*, De Boer & Belterman, 1980b). The karyotype of the latter species has a diploid number of approximately 76, including a single pair of large metacentric autosomes, two pairs of medium-sized and four pairs of small biarmed autosomes.

Due to the great inter-family heterogeneity among the four Coraciiform families studied to date, further investigations in this order, especially in the five remaining families, would be of great interest.

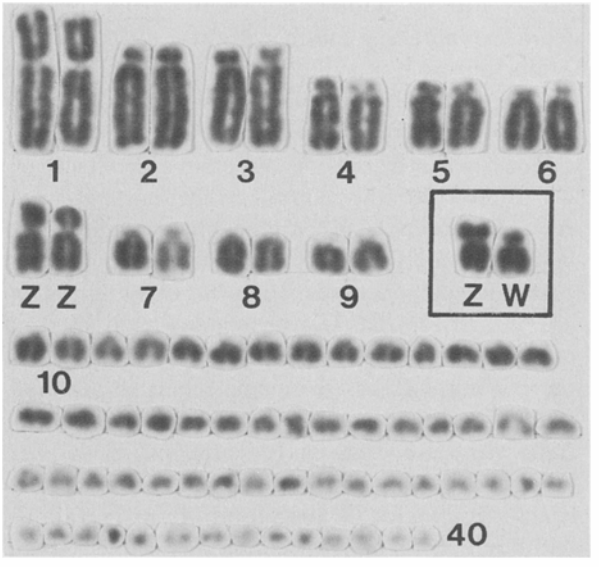


Fig. 62. Representative karyogramme of male *Cephalopterus penduliger* (Cotingidae). Inset shows ZW-pair of female.

Passeriformes

Three species of passeriform birds were studied by the present authors. Two of them are brought on record since they belong to families or subfamilies hitherto unstudied karyologically. The third one is shown for reasons of comparison. Due to the highly complex taxonomy of the Passeriformes and the absence of karyological data on some 25 of its 66 families it does not seem appropriate to enter a discussion here on the karyotaxonomy of the order, the more so since in contrast to the three species studied by us, almost 200 have been studied by others (for references see De Boer, 1984). Thus, here the karyotypes are only briefly described and merely compared to those of near relatives.

Cephalopterus penduliger is the first representative of the Cotingidae studied cytologically. Its karyotype (Fig. 62) consists of approximately 82 chromosomes and shows a clear distinction between macro- and micro-autosomes. Pair 1 is made up of large submetacentrics. Pairs 2 and 3 consist of large subtelocentrics of almost equal size, which possess minute short arms. Pairs 4–6 are of medium size and subtelocentric with high arm ratios; the presence of their tiny short arms can only be observed in metaphases with very long chromosomes. The remaining autosomes are acrocentric in as far as their centromeric positions are detectable. They gradually decrease in size from small to minute. The Z-chromosome is submetacentric and has the same size as pairs 4–6. The W is not much smaller than the Z and submetacentric. Recently, a second species of the Cotingidae, *Pachyrhamphus castaneus*, was studied by De Lucca & Waldrigues (1983, in press), but details on its karyotypic structure are not yet available.

The primitive songbirds (non-Oscines) to which the Cotingidae belong have been extremely poorly documented cytologically. Nothing is known of the karyotypes of the suborders Eurylaimi (family Eurylaimidae) and Suboscines (families Menuridae and Atrichornithidae) while of the suborder Clamatores only three (including the Cotingidae) of the 13 families were studied. Apart from *Cephalopterus penduliger*, the total number of Clamatores species whose karyotypes have been described in detail is only three. These are *Myrmotherula hauxwelli* (De Lucca, 1974a) and *Thamnophilus doliatus* (De Lucca & Chamma, 1977) of the Formicariidae, superfamily Formicaroidea, and *Knipolegus cyanirostris* (De Lucca & Chamma, 1977) of the Tyrannidae, which together with the Cotingidae and a number of other families belongs to the super-

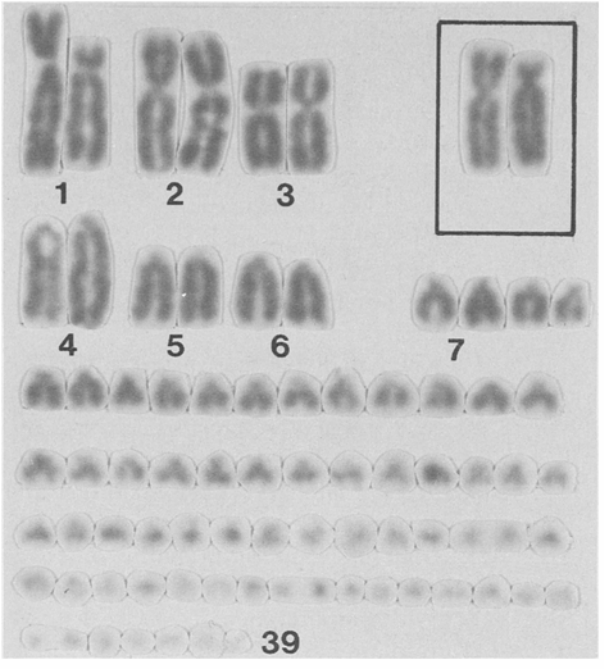


Fig. 63. Representative karyogramme of *Picathartes gymnocephalus* (Muscicapidae). This specimen probably is a male (pair 5 or 6 would represent the Z-chromosome pair when compared to Z-size in other Passeriformes). Note heteromorphism in pair 1. Inset shows pair 1 of another metaphase plate of the same specimen (note variability in length of long arm of subtelocentric homologue).

family Tyrannoidea (Shields, 1982, mentions a study of five species of *Empidonax,* family Tyrannidae, but gives no details on their karyotypic morphology). The karyotypes of the three species mentioned are different from each other as well as from that of *Cephalopterus penduliger* and do not offer starting points for a discussion on possible relationships.

Picathartes gymnocephalus is the first member of the Picathartinae (one of the many subfamilies of the Muscicapidae, suborder Oscines) karyotyped. Only one specimen of unknown sex was studied. This animal in all metaphase plates consistently showed heteromorphism in the first chromosome pair, one of the members being submetacentric (A.R. 2.3), the other subtelocentric (A.R. 4.1) (Fig. 63). Although the total length of the second homologue varied somewhat, it seemed clearly smaller than the first one, in particular the short arm seemed to be reduced in length. The possibility that this heteromorphic pair represents the ZW-pair of a female cannot be excluded. However, to date neither Z nor W-chromosomes of this large size have been found among birds. Therefore, it is more likely that we are dealing with heteromorphism of an autosome pair and that part of the short arm of the short homologue of pair one is translocated to a microchromosome. The supposed translocation product cannot be identified since all small to minute chromosomes are acrocentric and very gradually decrease in length. The karyotype includes five more pairs of macrochromosomes; two pairs of large biarmed chromosomes (pairs 2 and 3), one pair of large acrocentrics (pair 4) and two pairs of medium-sized acrocentrics (pairs 5 and 6). The latter two are the same length as most passeriform Z-chromosomes. The diploid number is approximately 78.

The Picathartinae are believed to be clearly related to the Timaliinae, a group in which they are sometimes included. Only two species of the latter subfamily have been studied cytologically, *Turdoides striatus* (Ray-Chaudhuri *et al.,* 1966; *Raman et al.,* 1978) and *Chrysomma sinense* (Sultana & Bhunya, 1980). Both, however, possess karyotypes which clearly differ from that of *Picathartes.* The same is true for three species of Sylviinae, another related subfamily (Bulatova, 1981), and for several tens of species of Turdinae and Muscicapinae, the only other subfamilies of Muscicapidae on which karyological data are available (for references see De Boer, 1984). Thus, *Picathartes* occupies a unique karyological position in this family.

Finally, the karyotype of *Corvus corone* consists

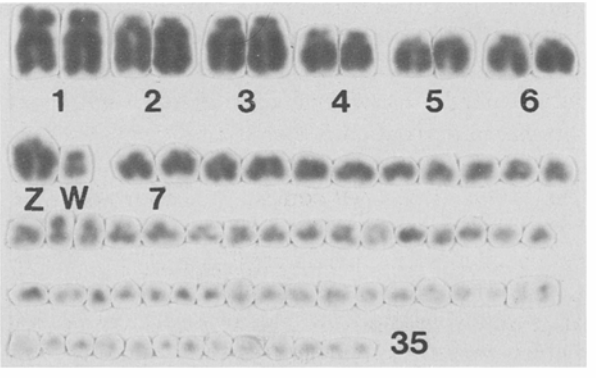


Fig. 64. Representative karyogramme of female *Corvus corone* (Corvidae).

of approximately 72 chromosomes. The first six pairs of autosomes are clearly distinguishable from the remaining small to minute acrocentric chromosomes. Pair 1 consists of large subtelocentrics (A.R. 3.5), pair 2 of large acrocentrics, pair 3 of large subtelocentrics with minute short arms (A.R. 10.0). Pairs 4 and 5 consist of medium-sized subtelocentrics with short arms only visible in metaphases with long chromosomes. Pair 6 consists of acrocentrics which are only slightly shorter than pairs 4 and 5. The Z is a subtelocentric chromosome of a similar size and shape to autosomes 4 and 5. The W is probably a small submetacentric chromosome. The karyotype of *C. corone* was recently studied by Ryttman & Tegelström (1981b), whose results for the species are identical to ours. The karyotype described above is also indistinguishable from those of *C. macrorhynchos, C. splendens* (Mittal & Sakhuja, 1980; Patnaik & Prasad, 1980), *C. brachyrhynchos* (Jovanovic & Atkins, 1969), and *C. corax* (Chromosome Atlas, 1973). *C. monedula* (Bulatova, 1981) probably differs from the other *Corvus* species by a clearly higher arm ratio of pair 1 and by a submetacentric Z-chromosome. This submetacentric pair 1 is also shared by four other species of the Corvidae, viz. *Cyanocitta cristata* (Jovanovic & Atkins, 1969), *Cyanopica cyana* (Bulatova, 1981) *Dendrocitta vagabunda* (Bhunya & Sultana, 1979; Patnaik & Prasad, 1980) and *Pyrrhocorax pyrrhocorax* (Bulatova, 1981). These species, with the exception of *Dendrocitta vagabunda* which shows a relatively large acrocentric Z, also share the medium-sized submetacentric Z-chromosome with *Corvus monedula.* For the remainder, the karyotypes of all corvids studied are identical. Thus, a karyotype such as that shown by *C. monedula* may be ancestral to the family, while subtelocentric pair 1 and acrocentric Z of *C. corone, C. macrorhynchus, C. splendens, C. brachyrhynchos* and *C. corax* may be shared derived characteristics.

Acknowledgements

The authors are greatly obliged to the curators of birds and their staffs of the zoological gardens in which they collected their material, viz. Antwerp (Mr R. van Boxcstaele), Arnhem (drs J. Wensink), Amsterdam (drs D. Dekker), Alphen aan de Rijn (Mr M. Verkade), Amersfoort (Mr M. Hoedemaker), Krefeld (Dr W. Enke and Dr A. Vogt), Rhenen (drs F. Engelsma), Walsrode (Dr B. Teichmann), Rotterdam (Ing. J. A. Assink and Mr K. Stusters), Wassenaar (Mr J. W. W. Louwman and Mr J. Rens) and Whipsnade (Dr V. Manton). Additional material was obtained from Mr S. van 't Hart (Rotterdam), Dr E. Nieboer (Amsterdam), Mr B. Ringelings (Voorst), Mr J. Raemakers (Nederweert), Mr J. van Dam (Polsbroek), Dr J. Docters van Leeuwen (Wamel), Mr J. van de Brink (Harderwijk), Mr B. Timmermans (Leersum) and Mr H. Weekes (Weert).

The following students participated in parts of the research work: C. van Geer-Plesman, R. P. Sinoo, M. W. M. van Dongen, A. Zonneveld, T. A. G. de Groen, G. Paulides and C. van der Ham. They are cordially thanked for their assistance and interest in the project.

Dr A. R. Glatston (Rotterdam Zoo) is acknowledged for correcting the manuscript, Miss I. M. Blanker for typing and retyping it. Mr F. A. C. M. Hollink (Rotterdam Zoo) prepared the drawn illustrations.

The Büttikofer Foundation (Rotterdam) is sincerely thanked for supplying the authors' laboratory with the equipment necessary for performing avian karyological research. The City of Rotterdam, finally, is acknowledged for financial support to the Rotterdam Zoological and Botanical Gardens in general, and to its Biological Research Department in particular. Without this invaluable support the application of modern techniques for sexing birds would not have been possible and breeding programmes involving many rare and endangered species would not have been as successful.

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Received 27.1.1984 Accepted 8.2.1984.