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SOMATIC CHROMOSOMES OF THE CONGO PEAFOWL (*AFROPAVO CONGENSIS*) AND THEIR BEARING ON THE SPECIES' AFFINITIES

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ABSTRACT.—The Congo Peafowl has an estimated diploid chromosome number of 76: nine pairs of macrochromosomes and approximately 29 pairs of microchromosomes. As far as the macrochromosomes are concerned, the karyotype closely resembles that of the Blue Peafowl (*Pavo cristatus*) and differs progressively more from those of guineafowl, domestic chicken, and various other gallinaceous birds. This evidence supports Chapin's view that *Afropavo* and *Pavo* are more closely related to each other than to any of the other galliform species. Karyotypic evolution within the order is discussed.

Since its remarkable discovery by James Chapin, the Congo Peafowl (*Afropavo congensis*) and its taxonomic affinities have been much discussed. Chapin (1936, 1937) believed the species to be most closely related to the Asiatic peafowl (*Pavo*), as is expressed in its generic name. His view that *Afropavo* represented an unspecialized, generalized or primitive peacock was adopted by Lowe (1938) on the basis of the latter's anatomical studies of the skeleton. Verheyen (1956), however, concluded that it was more closely related to African guinea fowl (Numididae), while Ghigi (1949) suggested affinities with the Himalayan Monal Pheasant (*Lophophorus impejanus*). Studies on the soluble proteins of the eye lens and of the skeletal, heart, and stomach muscles by Gysels and Rabaye (1962) and studies on the hind limb musculature by Hulselmans (1962) pointed to a rather isolated position for the Congo Peafowl. These authors recommended that this species be placed in a monotypic subfamily Afropavoninae of Phasianidae, remotely allied to *Pavo*. Finally, Benoit's (1962) work on Mallophaga showed the presence of ectoparasites on *Afropavo* that are related to those of the African guineafowl, as well as species related to those of the Asiatic phasianids.

In view of this taxonomic uncertainty, we readily accepted the opportunity to study the chromosomes of this species, which was offered by the Royal Zoological Society of Antwerp. We describe here the chromosome complement of the Congo Peafowl and discuss its taxonomic implications.

MATERIALS AND METHODS

Two Congo Peafowls, a cock and a hen, from the breeding stock of the Royal Zoological Society of Antwerp were made available for chromosome studies. Heparinized blood was aseptically drawn from the main brachial vein (V. basilica) and full-blood cultures were prepared according to techniques described by De Boer (1974, 1976). Pokeweed mitogen (PKW, Gibco 670-5360) and phytohaemagglutinin (PHA, Difco, 0528-56) were used as mitotic stimulators to induce cell division in lymphocytes. Cultures were harvested after three days of incubation at 40°C and 1.5 h of incubation in the presence of colchicine (0.0001% final concentration) as a metaphase arresting agent. Slides were prepared using the flame-drying technique and chromosomes were stained with acetic orcein (2%) and photographed using phase-contrast microscopy.

Arm ratios of individual chromosomes were calculated by dividing the length of the long chromosome arm by that of the short arm. In accordance with Levan et al. (1964), chromosomes with arm ratios between 1.0 and 1.6 are designated as metacentric, those with arm ratios between 1.6 and 3.0 as submetacentric and those with higher arm ratios as (sub)telocentric.

RESULTS

A total of 60 metaphase plates of Congo Peafowl were available for analysis. The diploid chromosome number for the species as estimated from these plates is 76. The karyotype consists of 9 pairs of macrochromosomes and of approximately 29 pairs of microchromosomes. Each of the macrochromosome pairs can be easily recognized individually. Pair 1 (see Fig. 1) consists of two large metacentric chromosomes (arm ratio 1.4) and pair 2 of somewhat smaller submetacentrics (arm ratio 1.6). The elements of pairs 3 and 4 are medium-sized subtelocentrics, those of pair 3 having minute short arms (arm ratio 7.9), while the short arms in pair 4 are clearly longer (arm ratio 3.2). The chromosomes of pair 5 are also subtelocentric (arm ratio 3.5), but they are considerably smaller than the preceding ones. Pairs 6 and 7 consist of small metacentric elements (arm ratios 1.5 and 1.2, respectively) which, in

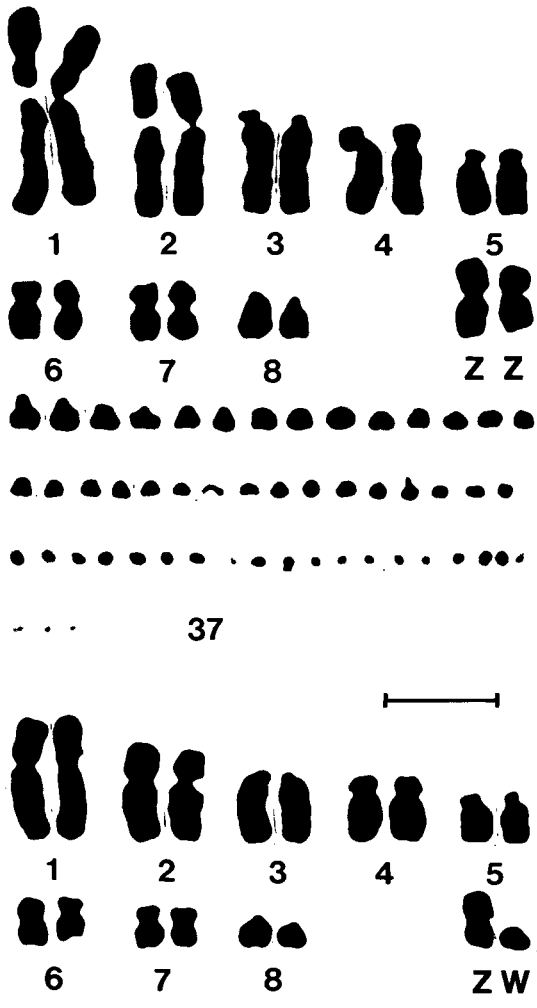

FIGURE 1. Representative male (top) and female karyograms of the Congo Peafowl. Of the female chromosome set, only the macrochromosomes and the sex chromosomes are shown. Bar indicates 5 μ of absolute length.

plates with more condensed chromosomes, cannot always be individualized with certainty. Finally, pair 8 consists of two small subtelocentrics with short arms of minute size (arm ratio 6.6). These elements are considerably longer than the longest microchromosomes, so that they can be recognized without difficulty.

The Z chromosome belongs to the macrochromosomes as well. It is metacentric (arm ratio 1.1) and of medium size, somewhat longer than the metacentrics of pairs 6 and 7. As in all bird species with the possible exception of the ratites, two Z chromosomes are found in the male, only one in the female. Instead of the second Z, the female chromosome complement carries a *W* chromosome. The avian *W* chromosome often can be identified by its specific staining properties (Bloom 1974). However, since we applied only routine staining techniques on our material of *Afropavo congensis* the *W* chromosome cannot be detected with any certainty because in this case it is indistinguishable from the microchromosomes. There are approximately 58 of the latter, all of which are subtelocentric or telocentric without apparent short arms. They gradually decrease in size until the smallest are barely visible using standard techniques. One of the largest microchromosomes was tentatively chosen as the possible female *W* chromosome.

In Figure 1 representative male and female karyograms are shown, while a schematic representation of morphology and relative lengths of the macrochromosomes is given in Figure 2.

DISCUSSION

In contrast to the situation in many of the other avian orders, the chromosome complements of the gallinaceous birds have been studied extensively. The karyotypes of almost 30 species have been described (for a review of avian chromosome cytology see De Boer 1981). In Figure 2, the macrochromosome pairs of *Afropavo congensis* are compared to those of some other galliform species which are relevant to the Congo Peafowl's taxonomic relationships.

As far as the macrochromosomes are concerned, the karyotype of *Afropavo* is virtually identical to that of the Blue Peafowl (*Pavo cristatus*; Ray-Chaudhuri et al. 1969; Sasaki et al. [1968] studied the karyotype of a *P. cristatus* × *P. muticus* hybrid). The only possible differences seem to be that the Z chromosome of the Congo Peafowl is somewhat smaller and more metacentric, while the Blue Peafowl's *W* chromosome is somewhat larger.

The karyotypes of Helmeted Guineafowl (*Numida meleagris*) and Vulturine Guineafowl (*Acryllium vulturinum*; Piccini and Stella 1970, Takahasi and Hirai 1974, Omura 1976) clearly differ from those of *Afropavo*. They lack a chromosome pair comparable to pair 5 in the Congo Peafowl and possess only one pair of small metacentric macrochromosomes. This pair possibly corresponds to pair 6 of *Afropavo*, while *Afropavo*'s pair 7 possibly is present as two separate telocentric chromosome arms among the larger microchromosomes of both guineafowl (indicated as 7q and 7p in Fig. 2).

The chromosome complement of the domestic fowl (*Gallus gallus* var. *domesticus*)

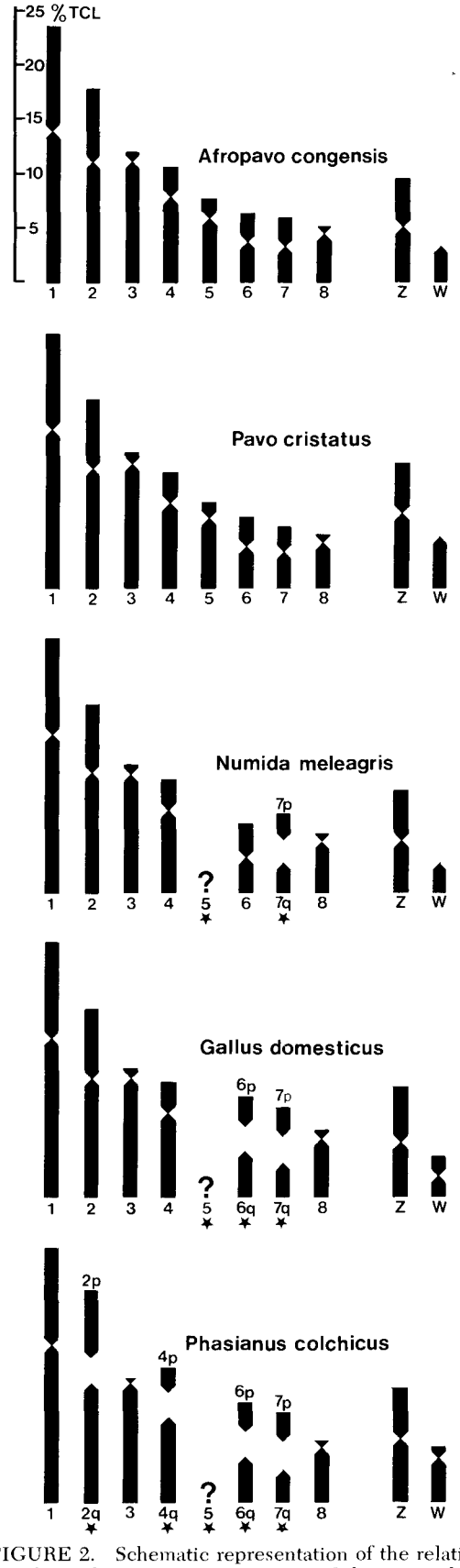

FIGURE 2. Schematic representation of the relative lengths and centromeric positions of the macrochromosomes and sex chromosomes of the Congo Peafowl (top). Each chromosome pair is indicated by a single bar, with a notch at the position of the centromere. The scale on the left indicates the percentage of the total haploid chromosome length (for methodology see De Boer 1974). For comparison, similar schemes of the macrochromosome and sex chromosome pair are given for four other gallinaceous species. Identical numbers indicate possibly homologous chromosome pairs. Separate chromosome arms are indicated with p for the short arm and q for the long arm. Stars indicate autosome pairs in each karyotype that differ clearly from those in *Afropavo*. The data on relative chromosome length and centromeric position of the chromosomes of *Pavo cristatus*, *Numida meleagris*, *Gallus domesticus*, and *Phasianus colchicus* are taken from the references mentioned in the text.

differs from that of *Afropavo* by the same features as do those of the guineafowl, notably the absence of pair 5 and the occurrence of the arms of the chromosomes of pair 7 as independent elements (e.g., Ohno 1961, Wang and Shoffner 1974, Stock and Mengden 1975). In addition, the second pair of small metacentric macrochromosomes of *Afropavo* (pair 6) is also absent in the domestic fowl. Possibly the chromosome arms of this pair are also present as independent elements among the larger microchromosomes of its karyotype (indicated as 6q and 6p in Fig. 2). Finally, the *W* chromosome of *Gallus* is metacentric instead of telocentric.

The karyotypes of the pheasants that have been studied so far are all identical and differ still more from those of *Afropavo*. As an example, the chromosomes of the Ring-necked Pheasant (*Phasianus colchicus*) are shown in Figure 2 (e.g., Stenius et al. 1963, Krishan and Shoffner 1966, Takagi and Sasaki 1974). As with domestic fowl, the pheasant karyotypes lack pairs 5, 6, and 7 of the Congo Peafowl but, in addition, pairs 2 and 4 are absent. Instead of these, three additional pairs of medium-sized telocentric chromosomes are present in the pheasants, which probably correspond to the long and short arms of pair 2 and the long arms of pair 4 of *Afropavo* (indicated as 2q, 2p, and 4q in Fig. 2). The short arms of pair 4 (4p) may be found among the pheasant's microchromosomes.

These comparisons show that in the series "peafowl, guineafowl, domestic chicken, and pheasants" there is a gradual increase in the numbers of karyotypic differences relative to *Afropavo*. The series begins with a karyotype almost identical to that of *Afropavo* and with many biarmed chromosomes and ends with karyotypes

with few biarmed chromosomes. Other gallinaceous birds in which chromosomes have been studied (cracids, turkeys, and many phasianids) possess low numbers of biarmed chromosomes, comparable to those in *Gallus* and the pheasants (De Boer and Belterman 1981, De Boer 1981).

Differences in the numbers of biarmed chromosome pairs between karyotypes of related species generally may be explained in two ways. First, two pairs of telocentric chromosomes may fuse to form one pair of biarmed chromosomes, thus reducing the number of telocentric chromosomes by four, the diploid number by two, and increasing the number of biarmed chromosomes by two. If chromosome fusion, a common evolutionary process (especially in mammals) occurs repeatedly, karyotypes evolve with gradually increasing numbers of biarmed chromosomes. Conversely, a fission in a pair of biarmed chromosomes resulting in the origination of two pairs of separate telocentric chromosomes (the original chromosome arms) would lead to a reduction of the number of biarmed chromosomes by two, and to an increase of the number of telocentric chromosomes by four and of the diploid number by two. Repeated fissioning, a process probably not uncommon in avian karyotypic evolution, would lead to karyotypes with ever decreasing numbers of biarmed chromosomes.

Thus, since chromosome fusion and fission are mechanisms with exactly opposite effects, they do not provide a clue as to whether those karyotypes with high or those with low numbers of biarmed chromosomes are the primitive condition in the Galliformes. In the first case, the karyotypes of *Afropavo* and *Pavo* would be the original ones and fissioning would have been the main mechanism leading to derived karyotypes with low numbers of biarmed chromosomes. In the second case, the karyotypes of the pheasants would be primitive and fusion would have been the predominant evolutionary mechanism leading to derived karyotypes with high numbers of biarmed chromosomes.

The fact that karyotypes with few biarmed chromosomes are found in most gallinaceous groups that have been studied might suggest that this condition was the original one. However, karyotypes with such low numbers of biarmed chromosomes as those of the pheasants, many of the smaller phasianids, and the Turkey (*Meleagris gallopavo*)—all of which lack a biarmed second chromosome pair—can hardly be considered as primitive, since this second chromosome pair is characteristic for many other groups of birds belonging to diverse orders (e.g., Pelecaniformes, Ciconiiformes, Phoenicopteriformes, Cathartiformes). Evolution predominantly by chromosome fusion must therefore be probably excluded. The finding of almost identical karyotypes in the Razor-billed Curassow (*Crax mitu*) and the domestic fowl (De Boer and Belterman 1981), both having a moderate number of biarmed chromosomes including the typical second chromosome pair, could indicate that this type of chromosome complement was the original one. This would suggest that karyotypic evolution in the Galliformes took place in two directions, one leading to the karyotypes with higher numbers of biarmed elements by fusion (*Pavo*, *Afropavo*, and the guineafowl), the other leading to karyotypes with lower numbers of biarmed elements by fission (many phasianids and meleagridids).

However, the picture is complicated by the uncertain taxonomic relationships of the various galliform groups. Some authors place turkeys, guineafowl, grouse and phasianids in a single family, Phasianidae, and retain only the cracids and megapodes as separate families. Sibley and Ahlquist (1972) even recommended the inclusion of the cracids in the Phasianidae on the basis of their studies on egg-white proteins. This would certainly have important implications with regards as to which karyotypic structure was the original in this order. If all the galliformes with the exception of the megapodes were to be included in a single family with uncertain within-group relationships, it is even possible that the original karyotype was one with a high number of biarmed chromosomes (comparable to those of *Afropavo* and *Pavo*).

Whichever of the possible explanations of the galliform chromosomal evolution—fissioning, fusion, or a combination of both—is accepted, however, there can be no doubt as to the relationship between *Afropavo* and *Pavo*. On the basis of their karyotypic structure these two genera are more closely related to each other than to any of the other galliform species. It is also tempting to accept that the guineafowl are somewhere intermediate to *Afropavo* and *Pavo* on the one hand and *Gallus* and the pheasants on the other. Nevertheless, it must be emphasized that all cytogenetic comparisons, based on chromosome material stained with conventional techniques, are speculative because interspecific chromosome homolo-

gies can never be fully ascertained. Modern differential staining techniques ("chromosome banding"), so far applied to very few avian species, allow much more detailed comparisons, but even then, any conclusion needs confirmation from additional evidence such as determination of chromosome homology via hybridization and cytochemical data.

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