Chromosomal changes in vertebrate evolution

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The immense variety of karyotypes found in extant species is unmistakable evidence that the process of evolution is associated with karyotypic change. The question whether the chromosome changes are a cause or a consequence of speciation has been debated intensely for many years and, as is often the case with biological problems, there has been no unequivocal answer. Evolution operates along different lines in different groups of organisms. In animals, reproductive biology and population structure are important factors influencing the rate of karyotypic change. Still, the most extreme chromosomal rearrangements are not necessarily found in the most specialized species.

A great number of chromosome banding techniques has made it possible to study chromosomes of vertebrates in great detail. Some applications of these techniques to problems of chromosomal polymorphism in relation to mammalian speciation are presented.

INTRODUCTION

The chromosomes are the vehicle of the genetic constitution and therefore of paramount interest for the understanding of evolutionary problems. The chromosomes are almost entirely composed of DNA and proteins, and in all higher organisms, eukaryotes, the general structure and chemical composition of the chromosomes are in good agreement. However, the amount of DNA varies in different organisms, and also the way in which the DNA is packed: the size, number and shape of the chromosomes varies considerably, from the small dot-like microchromosomes of birds to the giant salivary gland chromosomes in larvae of *Drosophila* - the famous fruit fly. Most animals have chromosome complements, karyotypes, which are characteristic for the species to which they belong.

I hope that the two pioneers of vertebrate chromosome research, Sasaki Makino and Robert Matthey, will agree that the immense progress in knowledge of vertebrate chromosomes during the last 25 years is mainly due to technical improvements. Below, just a few milestones in the development of vertebrate chromosome research will be mentioned.

In the beginning of the 1950s, chromosome techniques already in use for plants and insects were adapted to vertebrates, and particularly to mammals. With the application of squash techniques, it was possible in 1951 for the first time to obtain chromosome preparations of a modern standard in cells from ascites tumours in the rat (Makino 1951) and in the mouse (Hauschka & Levan 1951). In 1952 T. C. Hsu introduced hypotonic treatment of the cells prior to fixation, and by combining
hypotonic and colchicine treatments of cells grown in vitro, Tjio & Levan in 1956 succeeded in determining the correct chromosome number of man, 46. This was the beginning of a new era in chromosome research.

The idea to use cells grown in tissue culture for chromosome studies has proven most fruitful, and a new milestone in the history of chromosome techniques was passed in 1960, when Moorhead and collaborators introduced the blood culture technique. Under the influence of phytohemagglutinin, lymphocytes alter into a blast-like cell type that enters mitosis after 2-3 d in culture. Good chromosome preparations from a few drops of blood can nowadays be obtained in a large variety of fish, amphibian, reptilian, avian and mammalian species.

In the period 1955-1965 not only tissue culture techniques but also many different direct methods were developed for the study of vertebrate chromosomes in somatic as well as germ cells, and one of the pioneers in this field was Charles E. Ford.

In 1968-1970 the chromosome banding techniques were introduced by Caspersson and his coworkers, and these techniques have immensely increased our possibilities to identify individual chromosomes and parts of chromosomes. Some of the methods induce a pattern of cross-bands along the chromosome and each band is characterized by its stainability, width and location. The banding techniques have made possible detailed comparisons of the chromosomes of various species, and increased our chances of evaluating chromosomal changes in evolution.

In principle three main types of change have occurred during evolution, namely:

1. polyploidization, i.e. doubling of entire genomes;
2. gene duplication and deletion;
3. structural rearrangement of the chromosomes.

DNA CONTENT AND CHROMOSOME NUMBER IN CHORDATA

In table 1 the diploid chromosome numbers and DNA content per nucleus are listed for the main groups of the Chordata, just to give a rough idea of the great variation in chromosome number and DNA content existing both between and within the different groups. In spite of the fact that only few species have been investigated in certain groups, particularly in tunicates, lancelets and primitive fishes, some general conclusions can be drawn. There remains little doubt that genome size is under evolutionary control (Bachmann, Goin & Goin 1972b).

Tunicates, lancelets and fishes

The primitive chordates, the tunicates and lancelets, have little DNA, only 6 and 17 % respectively of that of man, whereas the Cyclostomata have 40-80 % (Atkin & Ohno 1967). That there is no general correlation between chromosome number and DNA content is demonstrated in the Cyclostomata; the hagfish Eptatretus stoutii has double the amount of DNA per cell nucleus compared with...
the lamprey *Lampetra planeri*, although their chromosome numbers are 48 and 146, respectively (Taylor 1967, Zanandrea & Capanna 1964). Fishes in general have less DNA per nucleus than mammals and the genome size of teleosts is on average only \( \frac{1}{2} \) of that of mammals (Ohno 1974). The range of variation is large among teleosts, as could be expected since, among living vertebrates, this group comprises the largest number of species (about 12000; Freeman 1972). One often finds advanced teleost species having a very small genome, only about 20% of the

### Table 1. Chromosome Numbers and Nuclear DNA Content in Chordata

<table>
<thead>
<tr>
<th>sub-phylum - class - sub-class - infra-class - order</th>
<th>chromosome number, 2n</th>
<th>DNA content, percentage of Homo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tunicata (tunicates)</td>
<td>8-40</td>
<td>6</td>
</tr>
<tr>
<td>Cephalochordata (lancelets)</td>
<td>32-38</td>
<td>17</td>
</tr>
<tr>
<td>Vertebrata (vertebrates)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agnatha (jawless fish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclostomata (hagfish and lampreys)</td>
<td>34-48, 76-168</td>
<td>80, 40</td>
</tr>
<tr>
<td>Chondrichthyes (cartilaginous fish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elasmobranchi (sharks and rays)</td>
<td>62-104</td>
<td>79-211</td>
</tr>
<tr>
<td>Holoccephali (chimaeras)</td>
<td>ca. 58</td>
<td>43</td>
</tr>
<tr>
<td>Osteichthyes (bony fish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcopterygii (fleshy-finned fish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crossopterygii (Latimeria)</td>
<td>?</td>
<td>80-100</td>
</tr>
<tr>
<td>Dipnoi (lungfish)</td>
<td>34-38</td>
<td>2289-4062</td>
</tr>
<tr>
<td>Actinopterygii (ray-finned fish)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chondrostei (bichirs and sturgeons)</td>
<td>36, ca. 112 - ca. 240</td>
<td>334, 50-70</td>
</tr>
<tr>
<td>Holostei (garpikes and bowfins)</td>
<td>ca. 68, ca. 46</td>
<td>40, 35</td>
</tr>
<tr>
<td>Teleostei (teleosts)</td>
<td>16-104</td>
<td>12-135 (34)†</td>
</tr>
<tr>
<td>Amphibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoda (legless amphibians)</td>
<td>24-42</td>
<td>100-400</td>
</tr>
<tr>
<td>Urodeia (newts and salamanders)</td>
<td>22-64</td>
<td>28-2700</td>
</tr>
<tr>
<td>Anura (frogs and toads)</td>
<td>14-104</td>
<td>20-300</td>
</tr>
<tr>
<td>Reptilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelonia (turtles and tortoises)</td>
<td>26-66</td>
<td>80-89</td>
</tr>
<tr>
<td>Rhynchocephalia (tuatara)</td>
<td>36</td>
<td>?</td>
</tr>
<tr>
<td>Squamata (lizards and snakes)</td>
<td>20-56</td>
<td>47-83</td>
</tr>
<tr>
<td>Crocodilia (crocodiles)</td>
<td>30-42</td>
<td>80-83</td>
</tr>
<tr>
<td>Aves</td>
<td>52-98</td>
<td>45-80 (51)†</td>
</tr>
<tr>
<td>Mammalia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prototheria (monotremes)</td>
<td>52-64</td>
<td>92-97</td>
</tr>
<tr>
<td>Theria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metatheria (marsupials)</td>
<td>10-32</td>
<td>74-115 (84)†</td>
</tr>
<tr>
<td>Eutheria (placentals)</td>
<td>6-62</td>
<td>56-167 (99)†</td>
</tr>
</tbody>
</table>

† Figures in parentheses indicate average values.
human genome. Apparently evolution and specialization in the teleosts may have been accompanied by loss of DNA (Hinegardner 1968). Among some groups of fishes, e.g. the Salmonidae, it is likely that high DNA content is associated with polyploidization. Species like trout and salmon, whitefish and graylings appear to be old autotetraploids which have progressed toward diploidization in various degrees (Ohno 1970).

Three species of lungfish have been studied: the Australian *Neoceratodus forsteri*, the South American *Lepidosiren paradoxa*, and the African *Protopterus aethiopicus*. They all have an exceptionally high DNA content in their cells, about 23, 35 and 41 times more than man, respectively (Pedersen 1971). The high DNA content of the lungfishes can hardly be due to polyploidization, since the genetic material is packed in rather few chromosomes, 34–38, which consequently are enormous in size. Ohno (1970) postulates that the ancestor of the lungfishes increased their genome size exclusively by tandem duplication, but in this case the increase passed the point of no return, the genome becoming ‘frozen’ while containing enormous genetic redundancy. In this context it is interesting to note that the genome of *Latimeria*, the forerunner of higher vertebrates, is far less than that of lungfish (Pedersen 1971), perhaps no larger than that of mammals (Vialli 1957, quoted by Ohno 1974). It is a pity that so far nobody has been able to study the chromosomes of this living fossil. Ohno (1974) points out that 48 acrocentric chromosomes, gradually declining in size, are present in a large number of teleost species representing 5 distantly related orders, and discusses the fascinating question whether this karyotype should be ancestral, not only to teleosts but to all vertebrates. However, the question has for the present to remain unanswered.

**Amphibians**

The amphibians are characterized by high DNA contents, and this is particularly true for newts and salamanders (Bachmann, Goin & Goin 1972a, Morescalchi 1973). Ohno (1970, 1974) argues that the extraordinarily large genomes possessed by lungfish and salamanders indicate that they belong to a common specialized branch of vertebrate evolution. This branch has never affected the main line but reached evolutionally dead ends. Bush (1975) concludes that accumulation of non-genetic DNA may actually be a form of evolutionary senescence that, unless reversed, might eventually lead to extinctions of such forms. In contrast to Ohno, Morescalchi (1973) claims that the resemblance in nuclear DNA content between the present Dipnoi and some Urodela may easily be interpreted as merely a convergence phenomenon due to adaptation to similar environmental requirements. Among all amphibia there is a positive correlation between high DNA content, large cell size and length of the larval period, and according to Morescalchi (1973) there is no correlation between the total quantity of DNA of the genome of a species and its phylogenetic position.

Frogs and toads have on an average much less DNA/cell than newts and salamanders, but the range of variation is very large in both groups. In some
species of frogs the high DNA content is undoubtedly correlated with polyplidization. Three species of South American frogs belonging to the subfamily Ceratophryinae (family Leptodactylidae) represent a diploid, tetraploid and octoploid state of evolution, having \(2n = 22\), \(44\) and \(104\), respectively (Beçak, Beçak & Rabello 1967), and a relative DNA content of \(1:2:4\) (Beçak, Beçak, Lavalle & Schreiber 1967). Ceratophrys dorsata having 104 chromosomes is an auto-octaploid species evolved from a diploid with 26 chromosomes. Polyplid frogs are known to occur in five families (Pipidae, Leptodactylidae, Hylidae, Bufonidae and Ranidae); Bogart & Tandy (1976) conclude that polyplidy is a general phenomenon in frogs and may appear in any genus. Still, speciation by polyplidization is exceptional in higher vertebrates.

Reptiles and birds

The genome size of reptiles and birds is smaller than in mammals and the range of variation in respect to DNA content is much less than in fishes and amphibia. Birds have around 50% of the DNA content of mammals; lizards and snakes have usually 60–70; crocodiles and turtles have 80–90%. Bachmann, Harrington & Craig (1972c) show very convincingly that groups with large genomes, like urodeles and lungfish, have few living representatives, while groups with small genomes, like teleosts and birds, have large numbers of species.

Reptiles and birds are characterized by great variation in chromosome size, and although it is customary to talk about macro- and microchromosomes, the borderline between these two groups is sometimes difficult to determine. There seems to be no fundamental difference between the composition of macro- and microchromosomes, apart from the self-evident fact that the centromeric region of microchromosomes occupies an exceptionally large proportion of the chromosomes.

The most ancient order of the Lepidosaurian stock of reptiles is the Rhynchocephalia with only one single relict species, the tuatara, which lives on New Zealand islets. The tuatara has 36 chromosomes (Wylie, Veale & Sands 1968, quoted by Gorman 1973), and although the details of its karyotype are unique, it is interesting to note that \(2n = 36\) seems to be the ‘basic’ number for both lizards and snakes (Gorman 1973). However, one has to be careful not to draw too extensive conclusions from karyological data alone, as exemplified in reptiles. The branching point between birds and crocodilians is regarded to be much more recent than that between crocodilians and other surviving classes of reptiles (Mayr 1969). Nevertheless, the similarities between snake and bird karyotypes are much greater than between crocodiles and birds. All 21 species of living crocodiles have been karyotyped (Cohen & Gans 1970), and their chromosomes are more similar to those of mammals than to birds. The similarity between snakes and birds concerns also their sex chromosomes. Heteromorphic sex chromosomes are the rule in birds and higher snakes, but have not been demonstrated in crocodiles. Lower snakes and some birds, e.g. the ostrich (Takagi, Itoh & Sasaki 1972), have
also no distinguishable sex chromosomes, like the majority of turtles and lizards. In snakes and birds the female is the heterogametic sex, in turtles and crocodiles we do not know which sex is heterogametic although male heterogamy, interpreted as an XX/XY sex chromosome system, has been reported in the turtle genus *Staurotypus* of the family Kinosternidae (Bull, Moon & Legler 1974). Among lizards the rule seems to be that the male is heterogametic: male chromosome heteromorphism has been confirmed in representatives of three families, whereas female heteromorphism only has been found in one species of a fourth family (Gorman 1973). Heteromorphic sex chromosomes must have arisen independently in different groups of vertebrates. Although more advanced than in fish and amphibia, the mechanism of sex determination in reptiles is still at a relatively primitive stage.

Among birds the chromosome number varies from 52 to about 98. The kestrel (*Falco tinnunculus*) has the lowest number with no clearcut subdivision into macro- and microchromosomes (Renzoni & Vegni-Talluri 1966), and the snipe (*Gallinago gallinago*) has the highest with 28 macro- and about 70 microchromosomes (Hammar 1970).

As we have seen, polyploid species occur among fish and amphibia, but when a well entrenched mechanism for sex determination evolved with sex chromosomes of different size, it became impossible to utilize polyploidization as a means of obtaining new gene loci. In higher vertebrates like birds and mammals gene duplication can only be accomplished by unequal crossing-over and other mechanisms leading to tandem duplication (Ohno 1970). A few triploid species of lizards exist but they reproduce parthenogenetically (Gorman 1973). Triploid individuals can be viable in birds (Ohno et al. 1963) but in mammals triploidy, as well as tetraploidy, is incompatible with viability.

**Mammals**

Among mammals the DNA content varies relatively little and most species have a value similar to that of man. The highest value, 167% of that in man, has been recorded in the aardvark (Benirschke, Wurster, Low & Atkin 1970), a species with only 20 chromosomes, and this is another example of a lack of correlation between high chromosome number and large amount of DNA. In mammals variation in genome size is mainly due to gain or loss of repetitive DNA sequences, visible as dark regions in C-stained chromosomes. According to Morris (1965) there are 4237 species of living mammals and of these approximately $\frac{1}{3}$ have been studied with reliable chromosome techniques (Matthey 1973a, b). Figure 1 demonstrates the range of variation in chromosome number, number of species, and percentage of investigated species in the various mammalian orders.

Since there has been some controversy about the taxonomic and phylogenetic position of the living monotremes, cytological evidence of their reptilian, avian or mammalian relations is particularly important (Sharman 1973). The karyo-
types of two monotreme species have been described. The platypus, Ornithorhynchus anatinus, has $2n = 52$ in both sexes (Bick & Sharman 1975) whereas the echidna, Tachyglossus aculeatus, has $2n = 64$ in females and $2n = 63$ in males (Bick, Murtagh & Sharman 1973). Their karyotypes are very similar in general type with a high number of metacentric chromosomes which cannot be grouped into macro- and microchromosomes. In their karyotypes the monotremes are thus more similar to placental mammals than to reptiles or birds, but their karyotypes are remarkable in certain respects. All surviving members of the egg-laying mammals, the platypus, the echidna and the giant echidna (Zaglossus sp.) are characterized by a complex multivalent formation at first division of male meiosis (Bick & Sharman 1975). This formation may be interpreted as composed of six partly nonhomologous autosomes, and apparently the sex chromosomes are also incorporated in the multivalent chain. However, many details of the sex chromosomes and mechanisms of sex determination of the monotremes remain unsolved, but like in other mammals the male seems to be the heterogametic sex. Bick & Sharman (1975) conclude: ‘The monotremes show little chromosomal affinity with marsupials (Sharman 1973). On the other hand the differences between the chromosomes of monotremes and those of therian mammals support the conclusions of Kermack & Kielan-Jaworowska (1971) that the living egg-laying mammals are the surviving representatives of the alternative (nontherian) branch.

![Figure 1. Range of chromosome number variation in the Mammalian orders.](http://rspb.royalsocietypublishing.org/)
of a mammalian dichotomy which took place before the close of the Jurassic period.'

The marsupials have low chromosome numbers, usually between 14 and 22 and the original number is most probably $2n = 14$ (Hayman & Martin 1974).

Among placentals more than $\frac{2}{3}$ of the species have chromosome numbers between 36 and 56, but because of the great diversity of karyotypes it is impossible to recognize a primitive or ancestral karyotype. The mammalian ancestor may have had 46 or 48 chromosomes, and the deviations from the original number may have been caused by centric fusions of one-armed and centric fissions of two-armed chromosomes. It is tempting to speculate about an optimal chromosome number: in general, a high chromosome number favours genetic recombination, a low number favours conservation of established linkage groups. As pointed out by White (1973b) the karyotypes must be adapted to the cellular dimensions of the organism in which they occur. Since the DNA content in mammals is relatively constant, chromosome size is inversely proportional to chromosome number. Few and large chromosomes may run into trouble at mitoses or meiosis due to limitation in length of the metaphase spindle, the chromosome arms would be too long to be pulled apart completely at anaphase. (However, the few and large chromosomes of the Indian muntjac demonstrate that this difficulty may be overcome.) A high number of small chromosomes may increase the risk for non-disjunctinal events with aneuploid cells or unbalanced gametes as a result.

Compared with amphibians, reptiles and birds, mammals have a wide range of variation in chromosome numbers. The lowest chromosome number among mammals, $2n = 6$, is found in a small deer from southern Asia, the Indian muntjac (Wurster & Benirschke 1970) and the highest chromosome number, $2n = 92$, has been reported for a fisheating rat from Peru, Anotomys leander (Gardner 1971). It is natural that the widest range of variation is found in the largest order, the Rodentia, but a small order like the Perissodactyla with only 16 species also shows great variation, from $2n = 32$ in the mountain zebra (Equus zebra) (Benirschke, Low & Heck 1964) to $2n = 84$ in the two African rhinoceros species (Diceros bicornis and D. simus) (Hungerford, Sharat Chandra & Snyder 1967; Hsu & Benirschke 1973).

**Karyotype stability and speciation**

The species is the fundamental biological concept, and evolution is intimately connected with speciation. The immense variety of karyotypes found in extant species is unmistakable evidence that the process of evolution is associated with karyotypic change. This leads to the question: are the chromosomal changes a cause or a consequence of speciation? This question has caused considerable controversy, and is difficult to answer unambiguously, mainly because evolution operates along different lines in different groups of organisms.

[48]
Chromosomal changes and evolution

Seals and whales

Among mammals, seals and whales have exceptional karyotype uniformity and stability in contrast to, for instance, insectivores and rodents (Árnason 1972). Twenty-two species of seals have been studied (70% of the living species) and their chromosome numbers vary between 32 and 36 (table 2). Árnason (1974a) has demonstrated convincingly with chromosome banding techniques that the 32 chromosome karyotype of true seals has been derived from the 34 chromosome karyotype by fusion of two chromosome pairs. A comparison of the karyotypes of the sea lions and the true seals shows that the majority of the chromosome pairs have identical or virtually identical G-band patterns, indicating a common evolutionary lineage, and strongly supporting the theory of a monophyletic origin of the Pinnipedia.

Table 2. Chromosome numbers in seals (Pinnipedia) and whales (Cetacea) (from Árnason 1974c)

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Number of species studied</th>
<th>Chromosome number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinnipedia</td>
<td>Otariidae (eared seals)</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Odobenidae (walrus)</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Phocidae (seals)</td>
<td>9</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Cetacea</td>
<td>Odontoceti (toothed whales)</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Mysticeti (whalebone whales)</td>
<td>4</td>
<td>44</td>
</tr>
</tbody>
</table>

The karyotype uniformity in cetaceans seems to be still greater; among them, however, only about 20% of the species have been studied (table 2). The chromosomes have been investigated in 17 species, 13 odontocetes and 4 mysticetes, all but 2 of which have 2n = 44 (Árnason 1974b). Detailed karyotypic similarity indicates a phylogenetic relation, because karyotypic similarities do not originate de novo among unrelated species. The karyotype does not respond to selective pressure in the same way as anatomical and physiological characteristics. The karyotypes of the toothed whales and whalebone whales show such great similarities that there can be no doubt that they have a common ancestor, and that, in other words, the evolution of the whales is monophyletic, not diphyletic (Árnason 1972, 1974b). It is fascinating to speculate about the reason why the karyotypes of the seals have remained practically unchanged for at least 20 Ma and those of the whales for 50 Ma. Árnason (1972) associated the karyotypic uniformity of Pinnipedia and Cetacea with two biological factors, namely their reproductive biology (late sexual maturity and small progeny), and their ecology (good mobility in an environment without delimited niches). These factors should contribute to extreme karyotype stability, because the chances are remote that two animals with the same chromosomal rearrangement will meet and become founders of a subpopulation with the new karyotype.
The conditions are exactly opposite in certain other groups of mammal, for instance small rodents and insectivores (Árnason 1972). In these orders karyotypic variation among species is often great, and sometimes also among populations within the species. Many cryptic or sibling species are distinguishable only on the basis of their karyotypes; they may be morphologically indistinguishable but biologically distinct species (White 1973b). Small rodents and insectivores are characterized by high reproductive rate (early sexual maturity, large number of offspring—in spite of short life span—and low competitive ability) and restricted vagility in an environment with delimited niches. The chances for a new chromosomal rearrangement to become established by inbreeding are great, and due to reduced fertility in the structural heterozygotes a barrier against animals with the original karyotype will immediately be established. This is what White (1968) called stasipatric speciation: ‘certain chromosomal rearrangements such as centric fusions and inversions arising within the area of occupation of a species and, under admittedly exceptional circumstances, initiating genetic isolation of an incipient species as a result of the lowered selective value of structural heterozygotes’ (White 1975).

Árnason’s conclusions concerning the relation between karyotype stability or variability, and reproductive and ecological factors have recently been supported by Bush (1975) and by Wilson, Bush, Case & King (1975). They particularly stress the importance of the social structuring of the populations for producing small effective population sizes and inbreeding. Bush compares chromosome evolution in true dogs and foxes. All dogs have $2n = 78$ and identical karyotypes whereas foxes have a wide range of chromosome numbers. Bush concludes that the reason for this difference in karyotypic stability between dogs and foxes must be sought in their ecology and social structure. Most large carnivores, including dogs, form social groups with extensive home ranges, whereas foxes do not form cohesive units beyond a permanent pair association. The chance for a chromosome rearrangement to become fixed in a small, isolated population founded by a single pair of foxes is considerably greater than in wide-ranging carnivores, such as dogs (Bush 1975).

Wilson et al. (1975) conclude that the rate of karyotype evolution in placentals has exceeded that in other vertebrates by a factor of at least 5; however, the rate of karyotype evolution has not been the same in all placentals. The bigger the animal, the slower is its karyotypic evolution, because larger mammals usually have higher mobility and much larger home ranges than small ones, and consequently limited ability to establish the small isolated populations necessary for the fixation of a new chromosome mutation. An exception from the rule that species with large body size have slow karyotype evolution is the horses. There are 7 living species of the genus Equus, and they all have different chromosome
numbers: 32, 44, 46, 54, 62, 64 and 66. The Mountain zebra (*E. zebra*) of southwest Africa has the lowest chromosome number and the wild horse of Central Asia (*E. przewalskii*) the highest (review in Short 1976). There is not only a considerable variation in chromosome number (2n) but also in the number of main chromosome arms (NF), indicating that other rearrangements than simple centric fusion/fissions have taken part. This wide range of karyotypes may be correlated to the social structure of the horses. Most species are subdivided into coherent family groups consisting of a stallion, several mares and their young and a certain degree of inbreeding must be common according to Wilson *et al.* (1975).

**The muntjacs**

There are 5 species of muntjacs: *Muntiacus muntjak, M. feae, M. roosevellorum, M. reevesi* and *M. crinifrons. M. muntjak* is divided by Whitehead (1972) into 15 subspecies, *M. reevesi* into 2, and for the remaining 3 species no subspecies are recorded. Ellerman & Morrison-Scott (1951) list the same 5 species of muntjacs and 2 subspecies of *M. reevesi*, but list only 8 subspecies of *M. muntjak*. The chromosomes have been studied in two subspecies of the Indian muntjac and one of Reeves's muntjac. *M. muntjak vaginalis* has 2n = 6 in the female and 2n = 7 in the male (the different chromosome numbers being due to the sex chromosomes, XX and XY1Y2, respectively). One female of *M. muntjak* captured near Kuala Lumpur, Malaysia, was found to have 2n = 8 (Wurster & Atkin 1972). A diploid number of 9 would be expected in the male of this subspecies. (The specimen was considered by the authors to belong to the race *M. m. muntjak* on basis of the geographic range given by Ellerman & Morrison-Scott (1951). According to the map of distribution in Whitehead (1972) it should belong to *M. m. peninsulare.*) Homology of the chromosomes of the two subspecies could not be ascertained and the X chromosome appears not to be translocated to the same autosome as in *M. m. vaginalis*. Wurster & Atkin (1972) conclude that the karyotype difference between the two subspecies is pronounced enough to predict synaptic incompatibility in meiotic division of a hybrid offspring, thus conferring sterility on the hybrid. Consequently the two subspecies should receive species status.

The karyotype of Reeves's muntjac is composed of 46 acrocentric chromosomes (Wurster & Benirschke 1967), and is thus very dissimilar to that of the Indian muntjac. The fact that two species with very similar external morphology, like the Indian muntjac and Reeves's muntjac, have completely different karyotypes has led Ohno (1974) to conclude that the karyotype can change drastically in a very short time without appreciable genetic consequence. Thus Ohno was ‘inclined to believe that karyotypic changes in evolution are to be regarded as neutral changes which accompanied speciation not because they were advantageous but because they were harmless’. On the other hand, the fact that the Indian muntjac comprises many more subspecies than Reeves’s muntjac indicates that the chromosomal rearrangements leading to reduction in chromosome number are connected with the formation of subspecies (species?). If karyological differences could be...
demonstrated among the different subspecies, the significance of chromosomal rearrangements for speciation within this genus should be supported. It would really be a fascinating task to study all the species and subspecies of muntjacs with modern chromosome banding techniques and follow the chromosomal rearrangements in detail. Such studies should certainly contribute to our understanding of the role of chromosomal changes in mammalian evolution.

How chromosomal mutations become stabilized

The problem of the stasipatric model of speciation is not so much the origin of chromosome mutations as their fixation in the population. Chromosomal rearrangements seem to arise spontaneously with a frequency of about 1 in 500 individuals (White 1973a), but the number of those surviving in evolution is probably only about 1 in $10^4$ or $10^5$ (White 1975). The majority of chromosomal rearrangements will be inviable or deleterious and will automatically become eliminated by natural selection. A minority of the rearrangements will carry adaptive gene combinations and may be established in spite of (and thanks to) the reduced fertility of the heterozygotes.

How structural rearrangements like translocations and inversions may accumulate in a random mating population, despite the mutation’s deleterious effect on the fertility of heterozygotes, has been discussed by Bengtsson & Bodmer (1976). They consider the effects of evolutionary factors such as random genetic drift, segregation distortion, viability advantage and recombination modification. The authors reach the conclusion that ‘in the absence of empirical data it is, unfortunately, impossible to separate the importance that direct and indirect selection for chromosome mutations have in the evolution of karyotypes’ but ‘that fixation of chromosome mutations by drift only occurs under special, and presumably very rare, circumstances’.

Structural Rearrangements of Chromosomes

Compared with frogs and birds, mammals have undergone unusually rapid anatomical evolution, and this may be correlated to the rate of chromosomal evolution, which is much more rapid in mammals than in frogs and birds. Gene rearrangements may be a key factor in the evolution of organisms, because new patterns of regulation may be achieved (Wilson, Sarich & Maxson 1974; Prager & Wilson 1975).

Survey of Rearrangements

The principal rearrangements of the chromosomes are inversions (peri- and paracentric) and translocations. Inversions, particularly paracentric, have been detected in a great number of dipteran species by the detailed banding pattern of the polytene chromosomes. In mammals it was practically impossible to detect paracentric inversions until the chromosome banding techniques were introduced, but examples of differences between or within species due to paracentric inversions
are still rare. Pericentric inversions seem to be more common, but not as common as expected before the era of chromosome banding. In some groups of mammals different taxa exhibit the same chromosome number, but a varying number of chromosome arms. The classic explanation for such a phenomenon was that the variable number of chromosome arms was achieved by pericentric inversions or unequal reciprocal translocations. C-banding has revealed that in several cases the short arms of biarmed chromosomes were composed of constitutive heterochromatin only, the G-banding has shown that the long arms were identical to telocentric chromosomes of related species. There is thus a difference in DNA content, but not in functional genetic material. The best example of this mechanism is found in rodents of the genus *Peromyscus*; 23 species have been studied and all have $2n = 48$, but the number of total chromosome arms varies from 56 to 96 (Pathak, Hsu & Arrighi 1973).

The commonest type of rearrangements in mammals is the so-called Robertsonian exchange. It has been discussed for a long time whether these translocations always involve breakage in the arms adjacent to the centromere of ‘acrocentric’ chromosomes, reunion of the long arms and loss of the centromeric fragment(s), or whether breakage and reunion within the centromere of true telocentric chromosomes do occur. The discussion has also concerned whether true telocentric chromosomes without a small second arm do exist or not (Levan, Fredga & Sandberg 1964; John & Hewitt 1968). There seems to be no doubt now that centromeres can be located terminally and that centromeres can fuse, with or without loss of minute centromeric components (Fredga 1972; John & Freeman 1975; Hsu, Pathak & Chen 1975). In addition, a metacentric chromosome can give rise to two functional telocentric chromosomes by dissociation within the centromere.

**Centric fusions**

Good examples of centric fusions concerns local populations of feral mice (*Mus musculus*) in valleys of the Alps, the best known being the tobacco mouse of Val Poschiavo with $2n = 26$ instead of 40 due to centric fusions of 14 telocentric chromosome pairs (Gropp, Tettenborn & Lehmann 1970; Gropp, Winking, Zech & Müller 1972). More recently two populations of mice were discovered in the Apennines of central Italy with only 22 chromosomes due to centric fusions of 18 pairs of telocentrics (Capanna, Civitelli & Cristaldi 1973). Only the smallest autosome pair and the sex chromosomes were unchanged. Chromosome G-banding, as well as crossing experiments between Apennine mice (‘CD’ and ‘CB’) and tobacco mice revealed that different telocentric chromosomes were combined into metaacentrics in the three populations. Only one metaacentric pair was identical in two populations and none in all three, and at meiosis of F1 hybrids a ‘super-multivalent’ configuration was demonstrated at diakinesis (Capanna *et al.* 1976).
Centric fissions

Perhaps the best example of spontaneous centric fission in a wild animal is the isolated population of root voles (*Microtus oeconomus*) in central Sweden, which have $2n = 31$ and 32 instead of 30, which is the chromosome number in animals from all other parts of the wide distribution of the species (Fredga & Bergström 1970).

Another example of karyotype differences probably due to progressive dissociation of metacentric chromosomes concerns the mole rats (*Spalax ehrenbergi*) of Israel (Wahrman, Goitein & Nevo 1969a). Four different karyotypes exist, $2n = 52, 54, 58$ and 60, and each karyotypic form has a distinct range of distribution. The mole rats are distributed elinically and parapatrically from north to south, along biogeographic regions of increasing aridity, and the chromosome numbers increase gradually from 52 and 54 in the north to 60 in the marginal population in the south. No considerable physical barriers separate the forms, but nevertheless very few hybrids are found in the contact zone between two chromosome forms (Wahrman, Goitein & Nevo 1969b). The relative rarity of natural hybrids, karyotype homozygosity and ethological barriers to reproduction between chromosome forms suggest that they should be regarded as sibling species (Nevo 1969). They are very similar morphologically, and electrophoretically tested proteins and immunological crossreactivity suggest close genetic relationships (Nevo & Shaw 1972, E. Nevo, private communication). On the other hand, clear differences in oxygen consumption were recorded between three of the chromosome forms. The basal metabolic rates decreased elinically from the humid north to the arid south (Nevo & Shkolnik 1974). Reproductive barriers involved mainly chromosomal rearrangements reinforced by ethological factors rather than gene mutations. These subterranean rodents seem to be a good example of stasipatric evolution.

Centric fusions or fissions in karyotype evolution?

In mammals centric fusions are generally regarded to be more common than fissions, but different opinions exist among scientists. Todd (1975), for instance, regards fissioning as the main chromosomal mechanism in the evolution of artiodactyl karyotypes, and regards $2n = 14$ as the original number for this large and diversified order. His conclusion is supported by the evidence that the ancestral diploid number of marsupials most probably is $2n = 14$ (Hayman & Martin 1974).

It is usually difficult or impossible to tell in what direction chromosome evolution has taken place, whether by fusions or fissions, and this dilemma will be exemplified in the mongooses (*Herpestinae, Viverridae, Carnivora*). There are at least 28 species of mongooses, 12 belonging to the genus *Herpestes* and the remaining scattered in 12 other genera, each comprising 1–3 species (Fredga 1972). The chromosomes have been studied in 17 species, 9 belonging to *Herpestes* and the rest to 8 other genera. All *Herpestes* species and the single species of the genus *Atilax* have an unusual sex chromosome mechanism: by translocation of an
original Y chromosome (or part of it) to an autosome, males of these species have one chromosome less than the females. All other mongoose species have a normal XY/XX sex chromosome mechanism. The standard chromosome number for the subfamily is 36, and the karyotypes of all studied species are very similar, except for the deviating sex chromosome mechanism in some species and differences in chromosome number in 3 *Herpestes* species: *H. pulverulentus* has 39/40, *H. sanguineus* 41/42 and *H. ichneumon* 43/44. All other *Herpestes* species have 35/36 chromosomes like *Atilax paludinosus* (which actually should be included in the genus *Herpestes*). Figure 2 shows two simplified phylograms, alternative (1) based on centric fissions and (2) on centric fusions. In (1) we start with a karyotype with 36/36 chromosomes, one X of original type (Ohno, Bečak & Bečak 1964) and one comparatively large Y, namely a karyotype similar to that of *Cynictis penicillata* of today. An autosome-Y translocation takes place early in the *Herpestes* branch,
and the size of the free Y becomes reduced in the other branch. Four centric fission events lead to the karyotype of *H. ichneumon*.

Alternative (2) starts with the unique autosome-Y translocation and gives rise to a free Y chromosome by some kind of dissociation after a series of centric fusions having established the 36 chromosome karyotype.

Of the two alternatives, (1) is the most likely one from a cytogenetic point of view, in spite of the fact that we here start with a hypothetical karyotype similar to that of the most specialized species, and end up with the karyotype of the most ‘primitive’ one. However, there is no general rule about chromosome number and morphology on the one hand and morphological specialization in general on the other. In favour of alternative (2) speaks the fact that *Herpestes* is the oldest genus: it has been in existence for about 30 Ma or longer than any other recent genus of the order Carnivora. *Herpestes lemanensis* is known from the Upper Oligocene of France and other species of *Herpestes* are known from the Miocene of Spain. Species of *Mungos*, *Crossarchus* and *Cynictis* have been described from the Pleistocene of Africa (Hinton & Dunn 1967). It seems reasonable to conclude that the Herpestinae originated in southern Europe and spread into southern Asia and into Africa, where now all recent species but the 8 oriental ones occur. Only one species, *Herpestes ichneumon*, still remains in southern Europe. The chromosome investigations included species inhabiting the extreme ranges of distribution of the genus *Herpestes*, from South Africa (*H. pulverulentus*) to the Malay Peninsula (*H. brachyurus*). Most likely the translocation which led to the establishment of the very unusual sex chromosome mechanism in *Herpestes* was a single event which occurred in a common ancestor. The wide-spread distribution of the genus indicates an ancient origin of the autosome-Y translocation. Of the two alternatives, centric fission or fusion, the author favours the first one.

The discussion above concerns only the alternatives centric fission and fusion. It is also possible that both types may alternate in the same lineage.

**Conclusion**

Polyploidization has probably played a rôle when the nuclear DNA content was increased during the early evolution of vertebrates from lower chordates; polyploidization has exceptionally occurred in fishes and amphibians. In the majority of vertebrates, however, variation in DNA content among and within classes, orders and families must be due to gene duplications and deletions. In reptiles, birds and mammals the amount of DNA is relatively constant within the classes, whereas in fishes and amphibians great variations are found. In many groups of fishes and amphibians low DNA amounts appear to be a feature of specialized species rather than of generalized ones.

In general, different species have different karyotypes, but in some orders, like seals and whales, many distantly related species have apparently identical karyotypes. Mammals have been undergoing unusually rapid anatomical evolution
and the diversity of living forms is remarkable. Chromosomal evolution has also been faster in placental mammals than in other vertebrates which is reflected in a pronounced karyotypic diversity. It seems likely that chromosomal rearrangements have played an important role in the speciation process of certain mammalian groups like small rodents and insectivores. These groups are characterized by high prolificity and restricted vagility in an environment with well delimited niches, factors predisposing to inbreeding. Under these conditions the chances of a new chromosomal rearrangement becoming established are great, and the reduced fertility of the heterozygotes will act as a barrier against animals with the original karyotype.

The commonest type of chromosomal rearrangements in mammals is centric fusion and fission. When just a few pairs of chromosomes are involved, fertility does not seem to be particularly affected, as shown in populations of the common shrew, *Sorex araneus* (Ford & Hamerton 1970). When, on the other hand, many chromosome pairs are involved, as in the tobacco mouse, the fertility of the hybrids with the common house mouse is severely impaired. There is a tendency towards similar structural changes to establish themselves in one chromosome pair after another within the karyotype. This principle is called karyotypic orthoselection by White (1973), and a good example is the Apennine mouse where all the telocentric autosomes but one pair have fused into metacentrics, very similar in size and shape. In spite of the immense variety of karyotypes there are certain restrictions: mammalian chromosomes similar in length are also similar in shape more often than would be expected on a random basis (Bengtsson 1975); also the position of the centromere is non-random (Imai 1975). There seems to be a system of organization of the eukaryotic chromosome determining the position of each gene in relation to the two prime organelles of the chromosome arm, the centromere and the telomere (Lima-de-Faria 1976). Thus the location of the genes for 28S and 18S ribosomal RNA (the nucleolar organizing constriction) is largely the same in relation to the centromere and the telomere in eukaryotes.

It is evident that the chromosomal changes during evolution have been strictly governed by rules which are, however, understood only fragmentarily at present. Fortunately, there is good hope that the battery of new chromosome banding techniques which are now available will considerably improve our chances of understanding the rules of chromosomal evolution.

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