# CHROMOSOMES AND SPERMATOZOA OF THE GREAT APES AND MAN

BY

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## DECLARATION

The composition of this thesis is that of the candidate. The candidate has acted as investigator for these studies and has been personally involved in the design, organisation and conduct of experiments. Contribution of technical assistance has been duly acknowledged.

Hector N. Seuanez, M.D.

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## PUBLICATIONS

The following publications have been produced in connection with this thesis.

(1) Gosden, J.R., Mitchell, A.R., Seuanez, H., and Gosden, C. The distribution of sequences complementary to Satellite I, II and IV DNAs in the chromosomes of chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla)and orangutan (Pongo pygmaeus). Chromosoma (Berl.) 63: 253-271 (1977).

(2) Mitchell, A., Seuanez, H., Lawrie, S., Martin, D.E. and Gosden, J.R. The location of DNA homologous to human satellite III DNA in the chromosomes of the chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), and orangutan (Pongo pygmaeus). Chromosoma (Berl) 61: 345-358 (1977).

(3) Seuanez, H.N., Carothers, A., Martin, D.E. and Short, R.V. Morphological abnormalities in the spermatozoa of man and the great apes. In press, Nature (London) (1977a).

(4) Seuanez, H., Fletcher, J., Evans, H.J., and Martin, D.E. A chromosome rearrangement in an orangutan studies with Q-, C- and G-banding techniques. Cytogenet.Cell.Genet. 17: 26-34 (1976a).

(5) Seuanez, H., Fletcher, J., Evans, H.J. and Martin, D.E. A polymorphic structural rearrangement in two populations of orangutan. Cytogenet.Cell.Genet. 17: 327-337 (1976b).

(6) Seuanez, H., Mitchell, A., and Gosden, J.R. The chromosomal distribution of human satellite III during meiosis. Cytobios 15: 78-84 (1976c).

(7) Seuanez, H., Robinson,J., Martin, D.E. and Short, R.V. Fluorescent ("F") bodies in the spermatozoa of man and the great apes. Cytogenet.Cell.Genet. 17: 317-326 (1976d).

(8) Seuanez, H., Mitchell, A., and Gosden, J.R. Constitutive heterochromatin in the Hominidae and four satellite DNAs in man and homologous sequences in the great apes. (in press - Proc. Joint Seminar in Genetics, Montevideo, Feb. 10, 1977b.

(9) Seuanez, H.N., Mitchell, A., Gosden, J.R., Fletcher, J. and Martin, D.E. The distribution of DNA sequences homologous to four human satellites in relation to chromosome bands in man and the great apes. Submitted for publication Cytogenet.Cell.Genet. (1977c).

## ABSTRACT

The chromosome complement of four species phylogenetically related to man, the chimpanzee (Pan troglodytes), the pygmy chimpanzee (Pan paniscus), the gorilla (Gorilla gorilla), and the orangutan (Pongo pygmaeus) have been analysed with chromosome banding techniques and compared to the human chromosome complement. This has shown remarkable homologies between species, and presumed mechanisms of chromosome evolution have been proposed. Chromosome heteromorphisms in the great apes have been compared to those found in human populations, and most of them affected the distribution or the amount of constitutive heterochromatin and/or brilliantly fluorescent material, a situation comparable to man where such variations have been established as chromosome polymorphisms. However, a balanced polymorphic structural rearrangement involving large segments of euchromatic material has been found in two populations of orangutan. This rearrangement consisted of two pericentric inversions, one inside the other, comprising an unusual kind of chromosome polymorphism in mammalian populations. Moreover, it showed that pericentric inversions, the most probable chromosome rearrangements in the phylogeny of the chromosomes of man and the great apes, might not necessarily be restricted by infertility barriers, but may spread successfully in the population.

The patterns of late replication of the chromosomes of the great apes and man have been compared, using BUdr as a thymidine substitute in the cell cycle. This has shown remarkable similarities in the patterns of late replication between species, and, as in the human/

human chromosomes, most regions of late replication in the chromosomes of the great apes corresponded to areas of positive G-banding. Q-, C- and G-banding as methods of demonstrating chromosome homologies between these species have been analysed in relation to the content of highly repeated satellite DNAs in man and homologous sequences in the great apes. This has shown that the banding patterns are not informative about these sequences, and that they must reflect a degree of chromosome organization due to DNA packaging rather than DNA composition. Finally, the phylogeny of the chromosomes of man and the great apes has been reconstructed in view of the findings presented in this work and of previous data in the literature. In this study, man and gorilla resembled each other more closely than to any of the other species studied, a finding that is contrary to the generally held view that man and the chimpanzee are the two most closely related species.

Comparative studies of the spermatozoa of the great apes and man were undertaken and showed that man was not unique in producing pleiomorphic spermatozoa, since this feature was also present in the gorilla. Moreover, the morphology of human and gorilla spermatozoa resembled each other so closely that on morphological grounds it was impossible to distinguish the spermiogram of these two species. Fluorescent ("F") bodies were detected in the spermatozoa of the African apes, although the distribution of such bodies did not resemble that of human spermatozoa, where the Y chromosome is usually visible. An analysis of the haploid DNA content of the great apes and man was undertaken by estimating the total dry mass of the sperm head in these species. Man showed the lowest DNA content, whilst the gorilla showed the highest; this latter

latter species also showed a higher variability in the haploid DNA content than all other species, including man. Diploid spermatozoa were also detected in the gorilla, in proportions similar to those found in man. These findings on spermatozoa are indicative of a closer relationship between man and gorilla than between man and the other hominoid apes. Moreover, the heteromorphism of spermatozoa in both human and gorilla semen samples makes it unlikely that clothing induced hyperthermia is the cause of pleiomorphic spermatozoa in man.

## INTRODUCTION

The fact that man like all other living beings is a product of evolution rather than divine creation was first recognized by Charles Darwin in his book "The Descent of Man" (1871). Such a conception of human origin was perhaps the most revolutionary challenge to scientific and philosophical thought, and this led to intense controversy when first presented. In his earlier book "The Origin of Species (1859) Darwin had first explained how evolution occurred, and how organisms had adapted successfully to their environments through natural selection or the survival of the fittest. The species as a taxon was no longer considered a population rigidly limited within static bounds. On the contrary, species were dynamic populations among which variation occurred continuously and upon which natural selection could act, thus permitting the eventual emergence of a new species. Data from comparative anatomy showing structural similarities between different species such as man and the great apes, was no longer an indication of a divine pattern of common organization of living beings, but an indication that such species had evolved from a common ancestor from which they had gradually diverged.

At the time Darwin presented his ideas on the origin of man there was no proof that any such ape-like human ancestor had ever existed. Late in the nineteenth century, Dubois made the first discovery of a long series of humanoid fossil forms which still continue to be found to the present time. The Pithecantropus erectus (1894) was clearly the first direct evidence that Darwin's ideas on the origin of man were correct. The first discovered human ancestor was neither like modern man nor like an ape, but a mixture of both. Since that time the/

the fossil record has provided abundant evidence of the lineage leading to man, and the first direct ancestors of man probably existed as long ago as five million years (Tobias, 1973). Darwin's theory of evolution was also reinforced when the science of genetics appeared as a leading biological discipline as a result of the diffusion of the works of Mendel. Mendel's principles which were formulated in 1865 were unknown to Darwin and to practically all scientists of the time, but at the turn of the century they were rediscovered and became widely accepted. When the mechanisms of inheritance became known, some theories of evolution such as that of Lamark appeared obviously unacceptable, but Darwin's theory became reinterpreted in the light of the new evidence. Mendel's conception of the nature and properties of genes, dominance and recessiveness, segregation and independent assortment, permitted a clear understanding of the mechanisms of variation within species. Species were no longer defined by structural parameters but were considered as breeding populations of individuals among which genes were exchanged. Each species had a genetic pool restricted to its own individuals which had been isolated from the genetic pool of other species. Natural selection was conceived as the predominance of a genotype resulting from favourable environmental conditions increasing the incidence of the favoured genes in the population.

The chromosomal theory modified the original postulates of Mendel, since genes were no longer conceived as abstract units of inheritance but as concrete elements contained inside visible nuclear structures. Whereas Mendel's postulates of dominance and recessiveness were still applicable to genes, segregation and independent assortment were found to apply more precisely to the behaviour/

behaviour of chromosomes. The mode of transmission of groups of genes inside chromosomes was then explained by linkage, and the less frequent production of recombinants by crossing over. The study of the chromosome complement of different species and of the meiotic cycles of many organisms led to two important discoveries: first, that each species had a distinct chromosome constitution (chromosome number and chromosome morphology): second, that a normal chromosome constitution was necessary for meiosis to occur undisturbed in an individual with sexual reproduction. If man, then, has emerged from an extinguished miocene ancestor from which the great apes have also evolved, what would this imply? It would imply that an original population of breeding individuals with the same chromosome constitution has split into different subpopulations which became reproductively isolated. The original genetic pool of our miocene ancestor was split into new genetic pools, each of which became isolated from the others. Permanent reproductive barriers must have been established between each subpopulation, and it is likely that chromosome rearrangements within the group might have played a significant role (Bobrow and Madan, 1973; de Grouchy et al, 1973), although we cannot yet prove whether chromosomal change was a cause or a consequence of speciation.

Some of these intriguing mechanisms can be envisaged if the chromosomes of the great apes, the chimpanzee (Pan troglodytes), the pygmy chimpanzee (Pan paniscus), the gorilla (Gorilla gorilla) and the orangutan (Pongo pygmaeus), are compared to the human chromosome complement. Although it is true that there is at present some information on the chromosome complement of these species much still remains to be found out. The vast majority of reports are based on very/

very few animals, since these species are endangered in their natural environment and the number of animals at zoos and research centres is generally small. Thus material for analysis is difficult to obtain and the amount of information on chromosome structure and chromosome heteromorphisms in ape populations is considerably less accurate than that of human populations.

This dearth of information makes it difficult to prove the presumptive chromosome mechanisms that might have played a role in speciation. In this study we have analysed relatively large numbers of animals and made comparative studies on the chromosomes of the great apes and man for the following purposes, (1) to look at chromosome heteromorphisms in ape populations and compare them to those frequently found in human populations, (2) to analyse the degree of chromosome homology between species as revealed by chromosome banding patterns in relation to the pattern of late DNA replication in the chromosome complement of the great apes and man, (3) to analyse chromosome homologies between species as demonstrated by chromosome banding patterns in relation to the distribution of highly repeated human satellite DNAs in man and homologous sequences in the chromosome complement of the great apes and (4) to establish what might have been the phylogeny of the chromosome complement of the great apes and man.

There is also much less information on the spermatozoa of the great apes than on human spermatozoa. This is due, obviously, to the difficulty in obtaining semen samples from these species. A second section of this study will present some findings on (1) the morphology of mature spermatozoa of four species of great apes, (2) the demonstration of brilliant fluorescent regions of the chromosome complement/

complement of the great apes in mature spermatozoa, and (3) estimations of total dry mass of the head of the mature spermatozoa of the great apes and man to quantify the haploid DNA content of each species.

## MAN AND THE CLASSIFICATION

Although there is agreement that man and the great apes belong to the superfamily Hominoidea together with the gibbons (Hylobatidae), the relationship between man and the great apes within the Hominoidea is a matter of discussion. According to Simpson (1945), the superfamily Hominoidea can be divided into two families, Pongidae and Hominidae (Table 1). This classification considers man as the only living species of the family Hominidae, whereas the great apes are included in the family Pongidae. This is based on data from comparative anatomy and evidence from the fossil record. The criteria to differentiate the Pongidae from the Hominidae are numerous but the most relevant are probably the following three: the cranial capacity (below 600 cc in the Pongidae, above 1300 cc in modern man), a body structure adapted for bipedal posture which is well developed in man, and the capacity of man (or primitive humanoids) to produce primitive tools.

A different classification proposed by Goodman (1974) based on biochemical and immunological distances between man and the great apes has been followed in the present study and is illustrated in Table 2. On molecular grounds, the phylogenetic divergence between man and the great apes is less apparent than from anatomical criteria. As an example, the aminoacid sequence of human and gorilla haemoglobin has been found to differ by only one aminoacid in each $\alpha$ and $\beta$ chain (Zuckerkandl and Schroeder, 1961). Findings of this kind have led Goodman (1974) to divide the Hominoidea into two families, Hylobatidae (gibbons) and Hominidae (man and great apes). Within the Hominidae, the subfamily Homininae includes the genera Pan, Gorilla/

TABLE 1

CLASSIFICATION OF SIMPSON (1945)

Superfamily HOMINOIDEA (Simpson 1931)
- Family PONGIDAE (Elliot 1913)
  - Subfamily PONGINAE (Allen 1925)
    - *Pongo* (Lacepede 1799)
      - *pygmaeus* (Linnaeus 1760).....Orangutan
        - *pygmaeus* (Linnaeus 1760).....Borneo orangutan
        - *abelii* (Lesson 1826).....Sumatra orangutan
    - *Pan* (Oken 1816).....Chimpanzee
      - *paniscus* (Schwarz 1929).....Pygmy chimpanzee
      - *troglodytes* (Blumenbach 1799).....Chimpanzee
    - *Gorilla* (I. Geoffroy 1852)
      - *gorilla* (Savage & Wyman 1847)
        - *gorilla* (Savage & Wyman 1847).....Coast gorilla
        - *beringei* (Matschei 1903).....Mountain gorilla
  - Subfamily HYLOBATINAE (Gill 1872)
    - *Hylobates* (Illiger 1811).....Gibbon
      - *agilis* (Cuvier 1821).....Dark-handed gibbon
      - *concolor* (Harlan 1826).....Black gibbon
      - *hoolock* (Harlan 1834)
      - *klossii* (Miller 1903).....Kloss's gibbon
      - *lar* (Linnaeus 1771)
        - *lar* (Linnaeus 1771).....White-handed gibbon
        - *pileatus* (Gray 1861)
      - *moloch* (Audebert 1797).....Sunda island gibbon
        - *mulleri* (Martin 1841)
    - *Symphalangus* (Gloger 1841).....Siamang
      - *syndactylus* (Raffles 1821)
- Family HOMINIDAE (Gray 1825)
  - *Homo* (Linnaeus 1758).....Man
    - *sapiens* (Linnaeus 1758)

TABLE 2

CLASSIFICATION OF THE HOMINOIDEA FOLLOWING GOODMAN (1974)

SUPERFAMILY HOMINOIDEA

FAMILY HYLOBATIDAE

SUBFAMILY HYLOBATINAE

| | |
|---|---|
| *Hylobates* | (gibbons) |
| *Symphalangus* | (siamangs) |

FAMILY HOMINIDAE

SUBFAMILY PONGINAE

*Pongo*

| | |
|---|---|
| *pygmaeus* | (orangutan) |

SUBFAMILY HOMININAE

*Pan*

| | |
|---|---|
| *troglodytes* | (chimpanzee) |
| *paniscus* | (pygmy chimpanzee) |

*Gorilla*

| | |
|---|---|
| *gorilla* | (gorilla) |

*Homo*

| | |
|---|---|
| *sapiens* | (modern man) |

Gorilla and Homo. The genus Pongo, less closely related to man than Pan and Gorilla is included in the subfamily Ponginae.

When the overall picture of the phylogenetic tree of the Hominoidea is examined it is apparent that the original trunk split approximately 40 million years ago with the emergence of the Hylobatidae and the Hominidae. Approximately 30 million years ago, the common ancestor of the Hominidae, a miocene creature probably represented by the Dryopithecinae split into two divergent branches, the Ponginae and Homininae. The line leading to man probably split from the Homininae 20 million years ago in the form of an ape-like creature, the Ramapithecus. The first clearly distinct direct ancestors of man, the Australopithecinae probably appeared five million years ago. A more evolved Australopithecine, Homo habilis (Leaky, 1973) was probably the first member of the genus Homo which appeared three million years ago. Homo erectus (formerly called Pithecantropus erectus) appeared one million years ago, and seems to be closer to modern man than Homo habilis, though no existing link has yet been found between them in the fossil record. The line towards mdoern man follows Neanderthal man who inhabited the planet approximately 300,000 years ago and Cro Magnon man who lived 40,000 years ago.

# SECTION I

## THE CHROMOSOMES OF THE GREAT APES AND MAN

# CHAPTER 1: COMPARATIVE STUDIES OF THE CHROMOSOMES OF MAN AND THE GREAT APES. REVIEW OF THE LITERATURE.

Since the time when Tjio and Levan (1956) and Ford and Hamerton (1956) first reported that the normal chromosome number of man was 46, thus ending a long standing controversy, human cytogenetics has developed considerably as a leading discipline. The substantial improvements in the methods of obtaining chromosome preparations in man permitted the study of other mammalian species, among which are man's closest living relatives, the great apes (*Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla* and *Pongo pygmaeus*). Practically nothing was known at that time about the chromosomes of these species, except for an early report of Yeager *et al* (1940) who had observed meiotic divisions in preparations of the testis of a chimpanzee (*Pan troglodytes*). Based on the number of observed bivalents, Yeager *et al* (1940) reported the diploid chromosome number of this species as 48. Twenty years later, Young *et al* (1960) confirmed these findings in the chimpanzee using chromosome preparations obtained from bone marrow. Later on, the chromosomes of the other species of great ape were studied for the first time (Chiarelli, 1961; Hamerton *et al*, 1961; Chiarelli, 1962), and their diploid chromosome number was also found to be 48. The karyotypes of the great apes were compared to that of man as a first attempt to study the phylogeny of human chromosomes (Chu and Bender, 1962; Bender and Chu, 1963; Hamerton *et al*, 1963; Klinger *et al*, 1963; Egozcue, 1969). An interesting comparison between the chromosomes of *Pan troglodytes* and man was made by McClure *et al* (1969, 1971) who first reported/

reported a chromosomal numerical aberration in a non human primate. The propositus was a new born chimpanzee that was a trisomic carrier of a small acrocentric chromosome (No. 22 in their classification). The clinical condition in the propositus resembled that of Down's syndrome in man, and the trisomic chromosome was recognized as identical to chromosome 21 in man. It was thus evident that a similar clinical condition was produced in two phylogenetically related species due to an identical kind of chromosomal aneuploidy. This was confirmed with Q-banding (Benirschke et al,(1974).

Comparative studies with chromosome banding techniques.

The development of chromosome banding techniques in the early seventies stimulated a number of workers to make detailed studies of the chromosomes of the great apes and to compare their banding pattern with that of the human chromosome complement (Borgaonkar et al, 1971; Pearson et al, 1971; Chiarelli and Lin, 1972; de Grouchy et al, 1972 and 1973; Turleau et al, 1972 and 1975; Bobrow and Madan, 1973; Dutrillaux et al, 1973; Egozcue et al, 1973 a, b; Khudr et al, 1973; Lejeune et al, 1973; Lin et al, 1973; Pearson, 1973; Turleau and de Grouchy, 1973; Warburton et al, 1973; Miller et al, 1974a; Bobrow, 1975; Dutrillaux, 1975, et al, 1975b, c; Seuanez et al, 1976a, b; Seth et al, 1976; Bogart and Benirschke, 1977, a, b).

G- and R-banding.

With G-banding techniques (Drets and Shaw, 1971; Sumner et al, 1971a,Gallimore and Richardson, 1973), and with R-banding techniques (Dutrillaux/

(Dutrillaux and Lejeune, 1971; Sehested, 1974), several degrees of chromosome homology were detected when the banding pattern of the human chromosome complement was compared with those of the great apes. However, most of the studies so far undertaken were complex and confusing since there were no standard criteria of nomenclature of the chromosomes of the great apes as existed for man. Moreover, there was no general agreement on the criteria of chromosome homology between species, and this led to different interpretations of the comparisons between the chromosomes of man and the great apes. This difficulty was still existing at the time this study commenced, but much has been clarified by the recent recommendations of the Paris Conference (1971); supplement (1975). These recommendations have been followed in this study, although we have suggested some minor modifications. On the other hand, we have added some information which was not precise in this report, and this is especially related to the Y chromosome of the great apes, and to the distribution of constitutive heterochromatin in these species.

An analysis of the G-, R- (and Q-) banding patterns of the chromosomes of man and the great apes has led us to suggest that the Hominidae, having emerged from a common stock, have suffered gradual chromosome change through rearrangement which can account for the existing differences between the chromosomes of the living hominoid apes and man. Such chromosome rearrangements, playing a role in the phylogeny of the Hominidae, have been envisaged by de Grouchy et al (1973) and Bobrow and Madan (1973) who suggested that pericentric inversions were the most probable kind of chromosome rearrangement within the group. However, it has been

suggested/

suggested (de Grouchy et al, 1973) that pericentric inversions, if appearing initially in a population, would most probably be restricted by infertility barriers in the heterozygous carriers, and they could rarely become an effective mechanism by which chromosome variation could result. This is the case for most pericentric inversions involving large euchromatic regions in mammals. However, a complex chromosome rearrangement involving two pericentric inversions has been found to be widespread in two populations of Pongo pygmaeus, and supplies good evidence that the proposed mechanisms of chromosome variation in the Hominidae may not in fact result in the selective disadvantage of the heterozygous condition (Seuanez et al, 1976a and b).

Q-banding.

A comparative analysis of the chromosomes of man and the great apes using techniques of quinacrine fluorescence as described by Caspersson et al (1968, 1969), has revealed that man and the African apes (Pan troglodytes, Pan paniscus and Gorilla gorilla) may show brilliant fluorescent regions in their chromosome complements (Pearson et al, 1971). Some of these regions have been visualized in interphase nuclei (Egozcue et al, 1973a), and also in mature spermatozoa as fluorescent ("F") bodies, (Seuanez et al, 1976d). Pongo pygmaeus does not show brilliant fluorescence in its chromosome complement, and neither do two other species of Hominoidea, Hylobates lar and Hylobates moloch (Pearson et al, 1971; Tantravahi et al, 1975), two more distant living relatives of man. This led Pearson (1973) to postulate that brilliant fluorescence has probably appeared in the common ancestor of man and the African apes (the Homininae) after/

after the Ponginae split from the common stock, in coincidence with the generally held view (see Goodman, 1974) on the phylogenetic origin of the Ponginae.

C-banding.

With the C-banding technique as a method of demonstrating constitutive heterochromatin (Arrighi and Hsu, 1971; Sumner, 1972), a basic difference is observed between man and Pongo pygmaeus, on the one hand, and Pan troglodytes, Pan paniscus and Gorilla gorilla, on the other. These three species show terminal heterochromatic regions at the telomeres of many chromosome arms, but these regions are absent in the corresponding homologous chromosomes of man and Pongo pygmaeus. The significance of this finding is unclear, but following the generally held view on the evolution of the Hominidae and on the nature of constitutive heterochromatin it is likely that it represents regions of low genetic activity which probably appeared in the common ancestor of Pan and Gorilla after they split from the line leading to man. We know that some of these regions are late replicating (see Chapter 5) and that none of them contains detectable amounts of highly repeated DNA sequences homologous to any of the four main human DNA satellites. In other heterochromatic regions of the chromosomes of Pan and Gorilla such sequences have been detected (Seuanez et al, 1977b, c). However, the significance of these terminal heterochromatic regions still remains obscure.

G-11 banding.

Other chromosome banding techniques have been used to analyse the/

the chromosomes of man and the great apes, and to compare their karyotypes. Bobrow et al (1972) and Gagne and Laberge (1972) described a method for the demonstration of segments of human constitutive heterochromatin using Giemsa staining at a highly alkaline pH (G-11 technique). This procedure is especially useful for demonstrating the heterochromatic regions of chromosome 9 in man, although the technique also stains other heterochromatic regions of the human chromosome complement. When used to stain the chromosomes of Pan troglodytes (Bobrow and Madan, 1973; Lejeune et al, 1973), Gorilla gorilla (Bobrow et al, 1972; Dutrillaux et al, 1973; Pearson, 1973), and Pongo pygmaeus (Dutrillaux et al, 1975c) these species showed larger regions of G-11 staining than man. It has been postulated that the technique demonstrates satellite III rich regions in man and in the great apes (Bobrow and Madan, 1973). The relationship between banding and the distribution of satellite DNAs in man and homologous sequences in the great apes has been analysed in detail (Seuanez et al, 1977b and c, see Chapter 6 of this Thesis). This comparison indicates, however, that none of the presently used Q-, G-, C-, R- and G-11 banding techniques is unequivocally informative on the nature of the underlying chromatin. This is coincident with the generally held view that chromosome banding reveals a degree of chromosome organisation that is rather related to DNA packaging, and only indirectly to DNA composition.

T-banding.

Other methods of chromosome banding such as T-banding (Dutrillaux, 1973) have also been used. T-bands are produced by heat/

heat denaturation using a solution of Giemsa phosphate buffer in distilled water, and slides are then stained with acridine orange, a fluorescent dye, after decoloration. Under the fluorescent microscope, positively stained regions appear green. Most T-bands are located at the telomeres of the chromosome arms in man, but this technique has been used to analyse the chromosomes of the great apes (Dutrillaux, 1975).

Ammoniacal silver (Ag-AS) staining and the location of 18S and 28S rDNA cistrons.

The Ag-AS staining technique (Howell et al, 1975; Goodpasture and Bloom, 1975) has been found to be a very reliable method of demonstrating nucleolus organizer regions (NORs) in many species. This is evident when the results of Ag-AS staining (Goodpasture and Bloom, 1975) are compared with the location of 18S and 28S cistrons, using techniques of in situ hybridisation in the same species (Hsu et al, 1975). In man, the 18S and 28S cistrons have been located with techniques of in situ hybridisation at the secondary constrictions of the acrocentric chromosomes (Henderson et al, 1972 and 1973; Evans et al, 1974a, and the same sites can be demonstrated with Ag-AS staining (Tantravahi et al, 1976). In the great apes, the location of the 18S and 28S cistrons has also been reported (Henderson et al, 1974 and 1976a) and there is a good correlation between the sites of positive hybridisation and of positive Ag-AS staining (see Tantravahi et al, 1976). In man, Pan troglodytes and Pan paniscus all the chromosomes that contain the 18S and 28S cistrons are acrocentric and participate in satellite association. In Gorilla gorilla, however, there is no relationship between ribosomal cistrons and satellite association. A group of large acrocentric chromo-

somes/

chromosomes participate in satellite association although they show undetectable amounts of rDNA cistrons and do not stain positively with the Ag-AS technique (Henderson et al, 1976a; Tantravahi et al, 1976).

5S rDNA cistrons.

The 5S ribosomal genes in man detected by techniques of in situ hybridisation are located at the terminal region of the long arm of chromosome 1 (Steffensen et al, 1974; Atwood et al, 1975). In the great apes, chromosome 1 has a recognizable homologue in the four species, except that the arm ratio in chromosome 1 of the ape is the opposite to that of man. In chromosome 1 of the ape there is no secondary constriction, thus, the long arm of the human chromosome 1 is homologous to the short arm of chromosome 1 in the apes, and the short arm of chromosome 1 in man is homologous to the long arm of chromosome 1 in the apes. The 5S ribosomal cistrons in the great apes are placed at the terminal region of the short arm of chromosome 1, thus at the same site as in man (Henderson et al, 1976b).

Satellite DNA sequences.

Other kinds of highly repetitive DNA sequences have been localized in the chromosome complement of man and the great apes. Britten and Kohne (1968) found that the genome of many higher organisms may contain a substantial proportion of highly repetitive DNA. Some of these sequences can be isolated from the main band DNA as lighter or heavier fractions, or satellite DNAs (Kit, 1961). These sequences are not apparently

apparently transcribed, and their function still remains obscure. In man, four main satellites have been isolated (Corneo et al, 1973), and unlike other species, they represent a very small percentage of the total genome. These sequences have been located in the human chromosome complement using techniques of in situ hybridisation. Jones and Corneo (1971) and Jones et al (1973a,1974) detected satellite DNA sequences in the human chromosome complement and found that the secondary constrictions of chromosome 1, 9 and 16 were rich in these highly repetitive DNAs, as well as other regions such as the centromeres of the acrocentric chromosomes. Evans et al (1974b)found that the Y chromosome in man was the major site of hybridisation for the four kinds of satellite sequences. These findings were confirmed later by Gosden et al (1975) using chromosome preparations which had been previously stained with quinacrine fluorescence before the incubation with the complementary RNA (cRNA), so that each chromosome pair and each chromosome region was unequivocally identified. A grain count was made in an attempt to quantitate the amount of cRNA hybridised as an indirect estimation of the total amount of satellite DNA detected. This analysis showed that each sequence type could be present in different proportions although many regions contained more than one kind of sequence.

Jones et al (1973b)and Jones (1976) detected homologous sequences to human satellite III DNA in the chromosome complements of Pan troglodytes, Gorilla gorilla and Pongo pygmaeus, showing that this sequence has been conserved in the Hominidae. Prosser et al (1973) showed that Pan troglodytes had a satellite DNA fraction (fraction A) that was almost identical to human satellite III.

Gosden/

Gosden et al (1977) and Mitchell et al (1977) have localized sequences homologous to the four main satellite DNAs in Pan troglodytes, Gorilla gorilla and Pongo pygmaeus. These studies permitted the precise recognition of the sites of positive hybridisation, since chromosomes were identified with quinacrine fluorescence prior to hybridisation. Chromosome identification permitted a direct comparison of the karyotypes of man and the great apes following the standard criteria of the Paris Conference (1971); supplement (1975). It would appear that Gorilla gorilla is the closest species to man in terms of the content of homologous sequences to the four satellite DNAs. It has also been postulated (Gosden et al, 1977; Seuanez et al, 1977b) that the original DNA sequence from which each satellite DNA has originated by amplification was present in the common ancestor of the Hominidae in a few or single copies, and that each kind of sequence has been independently amplified after speciation. This model of independent amplification is contrary to the hypothesis of Jones (1976) who has postulated that one kind of sequence (satellite II) has emerged in the line leading to man after man diverged from Pan troglodytes.

Methylated DNA sequences.

A different approach to the study of the nature of constitutive heterochromatin in man and the great apes has been reported by Miller et al (1974b), and Schnedl et al (1975). This method is based on the property of 5-methylcytosine to become detectable by the technique of immunofluorescence. Antisera to 5-methylcytosine capable/

capable of reacting with 5-methylcystydylic residues in single stranded DNA was used on chromosome preparations which had been denatured by U.V. light or by heat treatment. In man, the sites of antisera binding correspond to some regions of constitutive heterochromatin of the human chromosome complement (Miller et al, 1974b). It was suggested that satellite DNA was showing a higher amount of methylation than main band DNA as in the mouse (Salomon et al, 1969). Schnedl et al (1975) compared two species of great apes (Pan troglodytes and Gorilla gorilla) with man, and it was evident that man and Gorilla appeared to be more closely related to one another than either was to the chimpanzee. Both man and Gorilla showed large areas of antibody binding in some autosomes and in the Y chromosome, but no comparable regions were detected in the chromosome complement of Pan troglodytes, where the general binding level was low.

Comparative gene mapping.

The detection and localization of DNA sequences that are present in few or single copies in the genome of man and the great apes has been partially achieved using techniques of cell hybridisation. Hybrid cell lines (e.g.between mouse and man) can be formed by using attenuated Sendai viruses (Harris et al, 1966). Cells resulting from such experiments can be cloned, and lines can be obtained in which individual isolated chromosomes of man are incorporated into the mouse chromosome complement. If products of human structural genes are traced in these hybrid cells, a tentative chromosome assignment can be made. This method has been successfully used in mapping/

mapping the genes of man and the great apes, and the most recent findings have been summarized at the Baltimore Conference (1975). It is evident from these reports (see Finaz et al, 1975; Rebourcet et al, 1975; Orkwiszewski et al, 1976) that the homologous chromosomes of the Hominidae, as defined as those chromosomes of different species with similar morphology and banding patterns, generally carry the same structural genes. This indicates that the chromosomes, as functional linkage groups, have undergone few changes during the evolutionary process of the Hominidae, in spite of the probable chromosome rearrangements occurring within the group. It also gives good evidence that banding patterns are useful in demonstrating homologous linkage groups in the Hominidae.

DNA replication in chromosomes.

Studies of the pattern of DNA replication have been a useful approach for analysing the timing of DNA synthesis in relation to the cell cycle. The method of labelling chromosomes with a radioactive precursor (tritiated thymidine) was first introduced by Taylor et al (1957), and has been used to study the chromosome complement of many organisms, including man (German, 1962; Morishima et al, 1962; Gilbert et al, 1962; Moorhead and Defendi, 1963; Schmid, 1963; Kikuchi and Sandberg, 1964; Lima de Faria, 1964; German, 1966; Gianelli, 1970; Passarge, 1970). One of the most important findings in the days prior to the availability of banding techniques was that the asynchrony of DNA replication within a chromosome group permitted the presumptive identification of a pair of homologues, based on a specific pattern of labelling. It was also evident in man that the Y chromosome in males and one of the

X/

X chromosomes in females were late replicating in relation to the rest of the chromosome complement. Using similar methods, Low and Benirschke (1969) studied the late replication pattern of the chromosome complement of Pan troglodytes and compared it with that of man. Although some chromosomes of man and chimpanzee showed a very similar morphology, Low and Benirschke (1969) found considerable variations between the two species in labelling pattern.

DNA replication sites in relation to chromosome banding.

It was not until the development of chromosome banding techniques that a more detailed comparison of these regions became possible. Ganner and Evans (1971) and Calderon and Schnedl (1973) studied the patterns of DNA replication in relation to the banding patterns of the human chromosome complement. When Q-banding and autoradiographic studies were performed on the same preparations, it was evident that a high intensity of fluorescence was related to a pattern of late replication, except for one of the X chromosomes in females and for the regions of the secondary constrictions of chromosomes 1, 9 and 16. Those showed negative fluorescence but were nevertheless late replicating.

The demonstration of late replicating sites with BUdr.

The patterns of DNA replication were better understood when BUdr (5-bromodeoxyuridine) was used as a thymidine analogue. Although BUdr had been used in the past (Hsu and Somers, 1961; Palmer and Funderburk, 1965) its effect on chromosome structure was not fully understood until Zakharov and Egolina (1972) demonstrated/

demonstrated that chromosome regions incorporating BUdr exhibited increased despiralization. This finding was dose dependent and inversely proportional in time between BUdr incorporation and the onset of mitosis. In 1973 Latt reported that BUdr had the property of quenching fluorescence when the chromosomes were stained with Hoechst 33258, a fluorescent dye. Furthermore, he noticed that the incorporation of thymidine five hours before harvesting a cell culture that had undergone one round of replication with BUdr had the effect of enhancing fluorescence with Hoechst 33258 at the regions where thymidine was incorporated. Thus, the differential staining produced a banding pattern, the regions of brighter fluorescence being also those of late replication. These regions of late replication in general corresponded to regions of positive G-banding of the human chromosome complement; one exception being the secondary constriction of chromosome 9 which is negatively G-banded. Not all positively G-banded areas, however, were late replicating. The reverse procedure, i.e. the late incorporation of BUdr five hours before harvesting a cell culture grown in a medium with thymidine, produced a reverse pattern to that initially observed. Late replicating regions appeared clearer, showing quenched fluorescence, whilst early replicating regions appeared brighter (Latt, 1974). The procedure described by Latt (1973) was later modified by Perry and Wolf (1974) using Giemsa staining and 2 SSC denaturation which allows permanent preparations to be made. Chromosome regions incorporating BUdr appear faintly stained. This method permitted a more precise analysis of the chromosome structure, especially in relation to the occurrence of sister chromatid exchanges (SCE) in cells undergoing more than one round of replication with BUdr. The technique/

technique of Perry and Wolf (1974) was used by Grzeschik et al (1975) and by Epplen et al (1975) to demonstrate the late replicating pattern of the human chromosome complement with greater precision than was obtained using autoradiographic techniques. A comparison was made with the G-band pattern of the human chromosome complement, confirming the findings of Latt (1973) that regions of late replication corresponded generally to regions of positive G-banding.

There is no information on the patterns of late replication in the chromosome complement of the great apes, except for the initial work of Low and Benirschke(1969) using tritiated thymidine. Studies with thymidine and BUdr incorporation will be presented in Chapter 6 of this section.

# CHAPTER 2: THE CHROMOSOMES OF THE CHIMPANZEE (Pan troglodytes) AND THE PYGMY CHIMPANZEE (Pan paniscus).

## (A) Introduction.

The chimpanzee (Pan troglodytes) is the best known of the great apes and its geographical distribution covers large areas of equatorial Africa. The pygmy chimpanzee (Pan paniscus), a separate and rarer species of chimpanzee is also distributed in equatorial Africa, and is its closest relative within the great apes. The chromosomes of both species of chimpanzee have been reported on at different times. In 1940 Yeager et al established the diploid chromosome number of Pan troglodytes as 2n = 48. Studies of the chromosomes of Pan troglodytes were later reported by Young et al (1960); Bender and Chu (1962); Chiarelli (1962); and Hamerton et al (1963), and these showed homologies between the chromosomes of Pan troglodytes and other hominoids, a finding which was better demonstrated a decade later with chromosome banding techniques, (Turleau et al, 1972; Chiarelli and Lin, 1972; de Grouchy et al, 1972, 1973; Bobrow and Madan, 1973; Egozcue et al, 1973b; Lejeune et al, 1973; Lin et al, 1973; Pearson, 1973; Warburton et al, 1973; Seth et al, 1976). The chromosomes of Pan paniscus were initially reported by Chiarelli (1962) and Hamerton et al (1963). The number of reports on this species are considerably rarer and restricted to fewer animals, since this species is especially protected and difficult to obtain through Zoos and research centres. With chromosome banding techniques it was shown that the Y chromosome of this species was not brilliantly fluorescent with quinacrine staining (Borgaonkar et al, 1971) as was the Y chromosome of man and the gorilla (Pearson et al/

et al, 1971). Khudr et al (1973), Dutrillaux et al (1975b, and Bogart and Benirschke (1977 a and b) have shown that this species closley resembles Pan troglodytes, although some minor differences have been reported between their karyotypes. Detailed information on the chromosomes of Pan troglodytes (but not of Pan paniscus) is given in the report of the Paris Conference (1971); supplement (1975). Their recommended criteria will be followed in this study, and the karyotypes of Pan paniscus will be arranged in a similar way to that of Pan troglodytes.

(B) The normal karyotype of Pan troglodytes and Pan paniscus.

The two species have a diploid chromosome number of 48. In Pan troglodytes, the autosomes comprise 15 metacentric pairs, 6 acrocentric pairs and two subtelocentric pairs. The metacentric chromosomes can be divided in three groups according to their size: a group of large metacentrics (pairs 1 to 5), a group of medium sized metacentrics (pairs 6 to 11), and a group of small metacentrics (pairs 18 to 21). The acrocentric chromosomes can be divided into two groups: the large acrocentrics (pairs 14 to 17), and the small acrocentrics (pairs 22 and 23). The two pairs of autosomes not yet mentioned, No. 12 and No. 13 are subtelocentric. The X chromosome is a medium sized submetacentric and the Y chromosome is a very small subtelocentric chromosome, which is clearly distinguishable by being the smallest chromosome of the complement. The fundamental number in this species can be considered as 79 if the subtelocentric chromosomes and the Y chromosome are counted as one armed in an individual with a 48, XY constitution. The G-banded karyotype of Pan troglodytes is shown in Fig.2.1 and Table 2.1 shows data on relative chromosome/

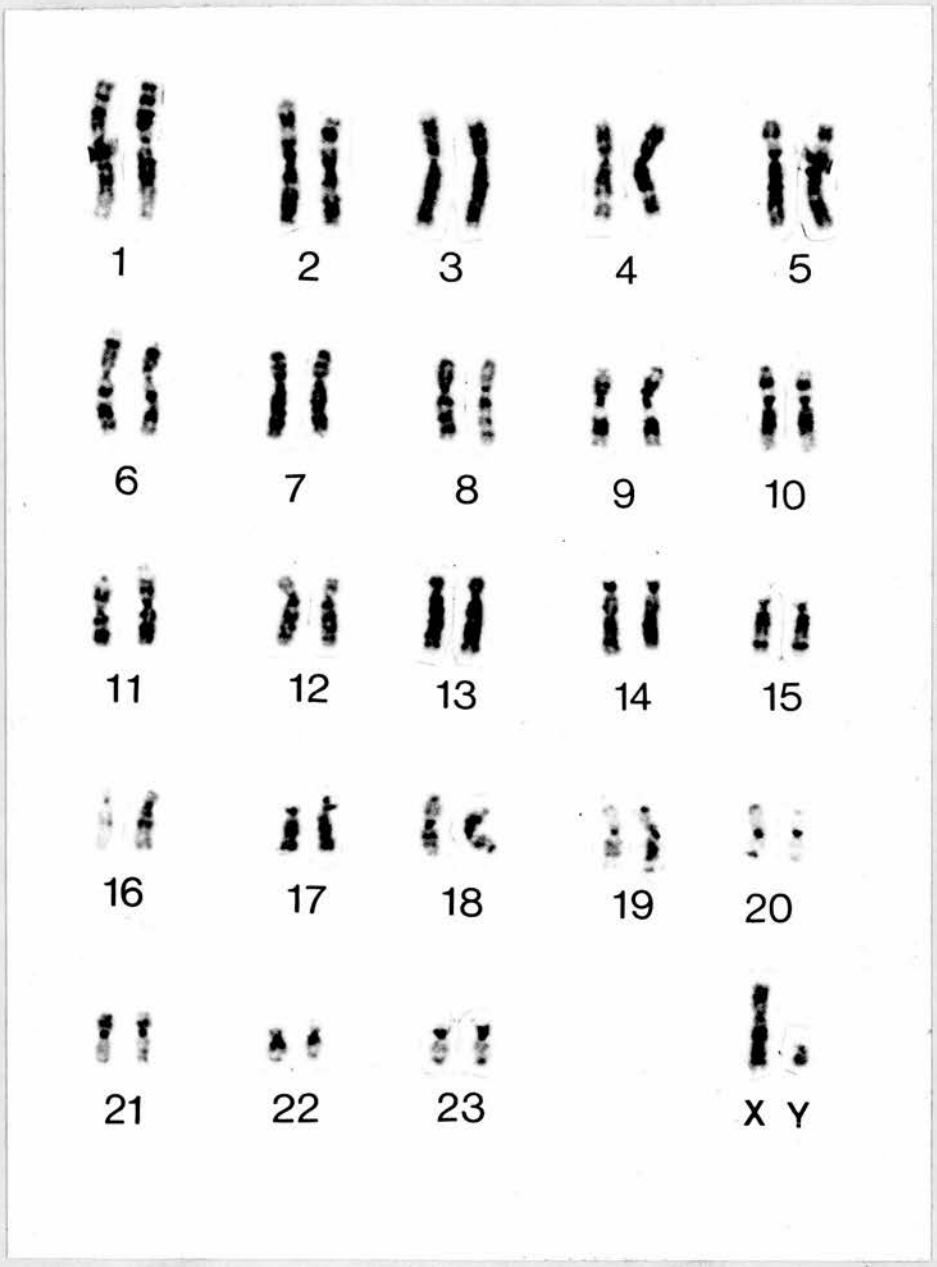


Fig.2.1. G-band karyotype of Pan troglodytes.

Table 2.1

RELATIVE LENGTH OF CHIMPANZEE CHROMOSOMES

| CHROMOSOME NUMBER | CHROMOSOME LENGTH (%) | SHORT ARM LENGTH (%) | LONG ARM LENGTH(%) | CENTROMERIC INDEX |
|---|---|---|---|---|
| 1 | 8.03± 0.66 | 3.55 | 4.48 | 44.2 |
| 2 | 6.87± 0.39 | 3.19 | 3.68 | 46.4 |
| 3 | 6.43± 0.35 | 2.30 | 4.13 | 35.8 |
| 4 | 5.88± 0.42 | 1.97 | 3.91 | 33.6 |
| 5 | 6.01± 0.35 | 2.20 | 3.81 | 36.6 |
| 6 | 5.80± 0.29 | 1.95 | 3.85 | 33.6 |
| 7 | 5.14± 0.18 | 1.59 | 3.55 | 30.9 |
| 8 | 4.59± 0.15 | 1.62 | 2.97 | 35.3 |
| 9 | 4.47± 0.35 | 1.68 | 2.79 | 37.6 |
| 10 | 4.49± 0.20 | 1.79 | 2.70 | 39.9 |
| 11 | 3.80± 0.03 | 1.40 | 2.40 | 36.8 |
| 12 | 4.25± 0.22 | 0.98 | 3.27 | 23.0 |
| 13 | 4.30± 0.16 | 0.92 | 3.38 | 21.4 |
| 14 | 4.12± 0.30 | 0.51 | 3.61 | 12.4 |
| 15 | 3.51± 0.32 | 0.46 | 3.05 | 13.1 |
| 16 | 3.11± 0.30 | 0.46 | 2.65 | 14.8 |
| 17 | 2.81± 0.32 | 0.50 | 2.31 | 17.8 |
| 18 | 3.46± 0.18 | 1.49 | 1.97 | 43.1 |
| 19 | 3.31± 0.16 | 1.42 | 1.87 | 42.9 |
| 20 | 2.82± 0.13 | 1.24 | 1.58 | 44.0 |
| 21 | 2.81± 0.33 | 0.47 | 2.34 | 16.8 |
| 22 | 1.79± 0.23 | 0.47 | 1.32 | 26.3 |
| 23 | 2.02± 0.24 | 0.36 | 1.66 | 17.8 |
| X | 5.21± 0.93 | 1.89 | 3.32 | 36.2 |
| Y | 1.10± 0.11 | 0.32 | 0.78 | 29.1 |

Numbers represent percentages of haploid autosome complement.

chromosome length and centromeric index.

In Pan paniscus the autosomes comprise 16 pairs of metacentrics 5 pairs of acrocentrics and 2 pairs of subtelocentrics. The metacentric pairs can be divided into a group of large chromosomes (pairs 1 to 5), of medium sized chromosomes (pairs 6 to 11), and of small chromosomes (pairs 18, 19, 20, 21 and 23). The acrocentric chromosomes comprise a group of larger chromosomes (pairs 14 to 17), and only one pair of small chromosomes (No.22). The subtelocentric pairs are Nos. 12 and 13. The X chromosome is medium sized and submetacentric; the Y chromosome is submetacentric and approximately the same size as chromosome 22. The fundamental number in this species can be considered as 81 if the subtelocentric chromosomes and the Y chromosome are counted as one armed in an individual with a 48, XY chromosome constitution. Table 2.2 shows data on the relative lengths and centromeric index of the chromosome complement of this species.

From Figs.2.1 and 2.2 it can be appreciated that the karyotypes of both species of chimpanzee have remarkable similarities, and that most of their chromosomes are identical. The following chromosomes, however, show differences which are well illustrated by G-banding; pairs 13, 14, 23, and the Y chromosome. The differences between the two species will be considered after analysing the respective Q- and C-banding patterns.

Q-banding.

Both species show very similar Q-banding patterns. In Pan troglodytes (Fig.2.3) there are 5 pairs of autosomes in which brilliant fluorescent regions can be found, i.e. in chromosomes No.14, 15, 17,

22/

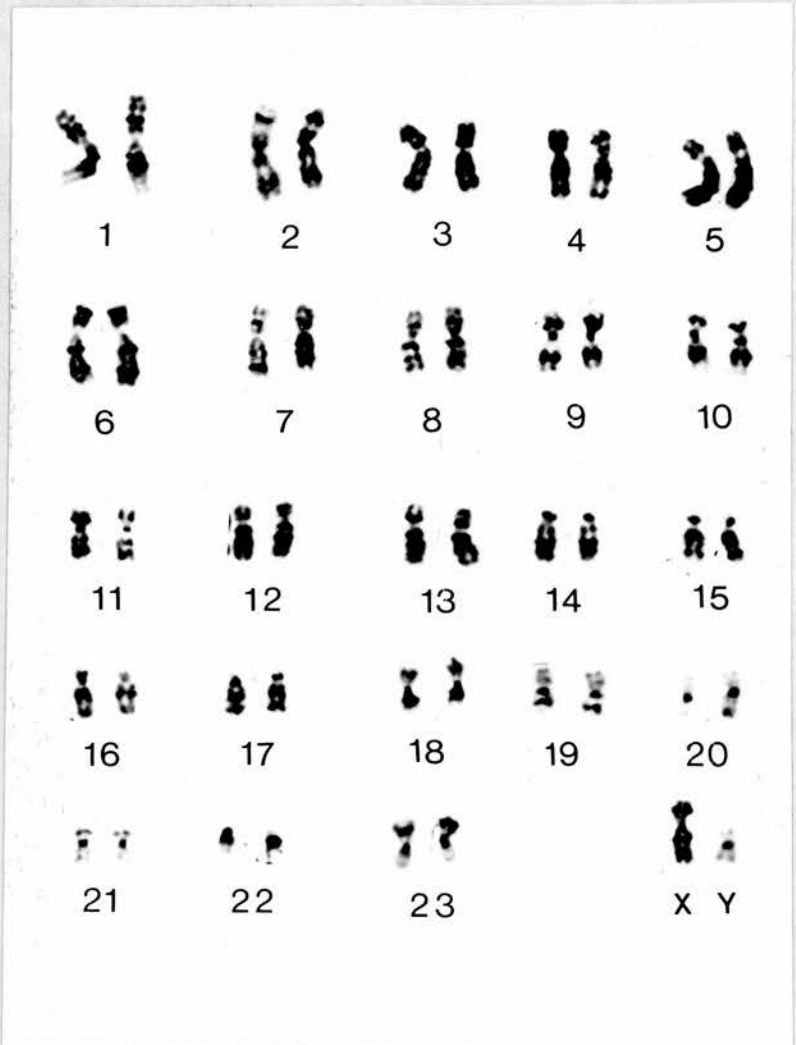


Fig.2.2 G-band karyotype of Pan paniscus.

Table 2.2

RELATIVE LENGTH OF PYGMY CHIMPANZEE CHROMOSOMES

| CHROMOSOME NUMBER | CHROMOSOME LENGTH(%) | SHORT ARM LENGTH(%) | LONG ARM LENGTH(%) | CENTROMERIC INDEX |
|---|---|---|---|---|
| 1 | 7.60± 0.13 | 3.21 | 4.39 | 42.2 |
| 2 | 6.45± 0.21 | 3.02 | 3.43 | 46.8 |
| 3 | 5.84± 0.55 | 1.99 | 3.85 | 34.0 |
| 4 | 5.59± 0.29 | 1.83 | 3.76 | 32.7 |
| 5 | 6.41± 0.63 | 2.32 | 4.09 | 36.2 |
| 6 | 6.03± 0.39 | 2.24 | 3.79 | 37.1 |
| 7 | 5.01± 0.13 | 1.80 | 3.21 | 35.9 |
| 8 | 5.01± 0.28 | 1.88 | 3.13 | 37.5 |
| 9 | 4.58± 0.26 | 1.82 | 2.76 | 39.7 |
| 10 | 4.51± 0.22 | 1.69 | 2.82 | 37.5 |
| 11 | 3.87± 0.12 | 1.59 | 2.28 | 41.0 |
| 12 | 4.09± 0.21 | 1.16 | 2.93 | 28.4 |
| 13 | 4.61± 0.21 | 1.27 | 3.34 | 27.5 |
| 14 | 3.74± 0.30 | 0.51 | 3.23 | 13.6 |
| 15 | 3.45± 0.56 | 0.56 | 2.89 | 16.2 |
| 16 | 3.11± 0.21 | 0.67 | 2.44 | 21.5 |
| 17 | 2.80± 0.30 | 0.46 | 2.34 | 16.4 |
| 18 | 3.28± 0.13 | 1.49 | 1.79 | 45.4 |
| 19 | 3.09± 0.33 | 1.53 | 1.56 | 49.5 |
| 20 | 2.93± 0.42 | 1.22 | 1.71 | 41.6 |
| 21 | 2.53± 0.29 | 1.04 | 1.49 | 41.1 |
| 22 | 1.92± 0.35 | 0.38 | 1.54 | 19.8 |
| 23 | 3.04± 0.41 | 1.43 | 1.61 | 47.0 |
| X | 4.82± 0.21 | 1.80 | 3.02 | 37.3 |
| Y | 2.14± 0.29 | 0.71 | 1.43 | 33.2 |

Numbers represent percentages of haploid autosome complement.

22 and 23. These correspond to the homologues of chromosomes 13, 14, 18, 21 and 22 in man. One acrocentric chromosome in Pan troglodytes (No.16) which is the homologue of human chromosome 15, shows in contrast to all the other acrocentrics, no brilliant fluorescence, and in fact no stalk or satellite region. This chromosome is never involved in satellite association as are all the other acrocentric chromosomes in this species. In the chromosomes in which there is brilliant fluorescence, it is always found at the proximal region of the short arm, but not at the terminal satellites. These stain pale or negatively with fluorescence. This contrasts with Gorilla gorilla (see Chapter 3) in which brilliant fluorescence is located at the satellites in the large acrocentric chromosomes.

In Pan paniscus, the regions which are brilliantly fluorescent are located in exactly the same chromosome pairs as in Pan troglodytes. (See Fig.9.12page 146-7).Lack of stalk and satellite region occurs in chromosome 16, identical to the situation found in Pan troglodytes. There is one chromosome pair, however, which, although carrying a brilliant fluorescent region, is morphologically different in Pan paniscus and Pan troglodytes. In the pygmy chimpanzee, chromosome No.23 is a small metacentric chromosome with a short arm in which a brilliant region is usually located at the distal half of the short arm. In Pan troglodytes, on the other hand, this chromosome is acrocentric with a stalk and terminal satellites.

C-banding.

In Pan troglodytes, all chromosomes have a C-band region at their centromere region. The centromere of chromosome 12 shows, however, a/

a very small region, which may occasionally pass unnoticed in insufficiently stained preparations. Fig.2.3 shows a C-band karyotype of a cell which was previously stained with quinacrine. Two findings are striking: (i) two chromosome pairs (No.6 and No. 14) show interstitial heterochromatic regions at their long arm, and (ii) terminal heterochromatic regions are found in the telomeres of many chromosome pairs. These are listed in Table 2.3. The acrocentric chromosomes show positive C-banding at their short arm regions, and the regions of brilliant fluorescence are stained positively with Giemsa together with the more distal region of the short arm (the stalk and the satellites). The Y chromosome shows a C-band only at its very small short arm. In Pan paniscus, the C-banding patterns resemble closely those of Pan troglodytes. The centromere regions of chromosome 1, 2 and 5 are somewhat difficult to visualize since the C-band regions are small. Chromosome 6 has an interstitial C-band at its long arm as in Pan troglodytes, but chromosome 14 does not show an interstitial C-band as in the latter species. The short arm of chromosome 23 is heterochromatic in Pan paniscus, as well as the short arm of the Y chromosome. The same regions of the same chromosome pairs in both species of chimpanzee show terminal C-bands. Thus, the information of Table 2.3 is valid for Pan paniscus as well as for Pan troglodytes.

(C) Differences between species.

Both species have a remarkable similarity in their karyotype if Fig.2.1 and 2.2 are compared. With G-banding, however, (Fig. 2.4) the short arm of chromosome 13 in Pan paniscus appears somewhat longer than in Pan troglodytes, as a result of the presence of an extra/

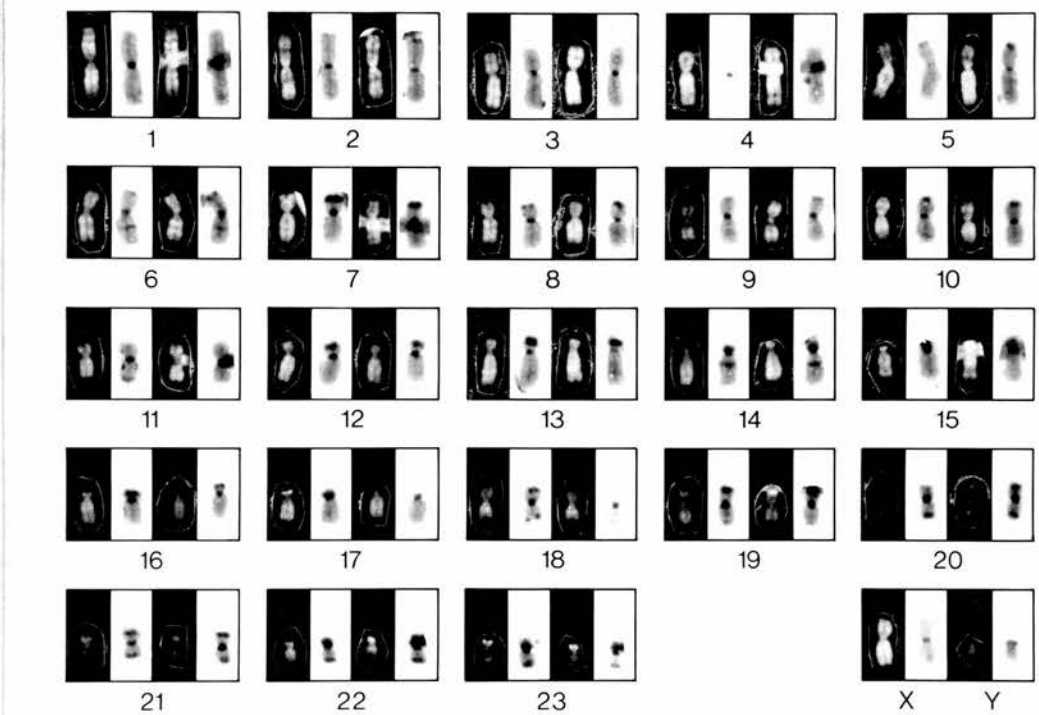


Fig.2.3. Q- and C-band karyotypes of Pan troglodytes.

TABLE 2.3.

Q- AND C- TERMINAL BANDS IN Pan troglodytes

| | p | q |
|---|---|---|
| 1 | - | - |
| 2 | + | - |
| 3 | - | - |
| 4 | - | - |
| 5 | + | - |
| 6 | + | - |
| 7 | + | - |
| 8 | + | - |
| 9 | + | - |
| 10 | + | - |
| 11 | + | - |
| 12 | + | - |
| 13 | + | - |
| 14 | + | - |
| 15 | + | - |
| 16 | + | - |
| 17 | + | - |
| 18 | + | + |
| 19 | + | + |
| 20 | + | + |
| 21 | + | + |
| 22 | + | + |
| 23 | + | + |
| X | - | - |

+ = positive banded region
- = negative banded region
p = short arm of chromosome
q = long arm of chromosome

extra positive G-band at the region above the centromere. This positive G-band was not found in the short arm of chromosome 13 in Pan troglodytes. The long arm of chromosome 13 of the two species had an identical G-banding pattern. The results observed with G-banding were confirmed when measurements of the chromosomes of the two species were made. The total length of chromosome 13 in Pan paniscus is slightly larger than in Pan troglodytes, but the length of the long arm of chromosome 13 in both species is almost identical (Table 2.1 and 2.2). The short arm of chromosome 13 in Pan paniscus is longer, and the centromeric index of this chromosome pair is higher than in Pan troglodytes. Chromosome 14 in Pan troglodytes shows an interstitial C-band (Fig.2.3) which is absent in the homologous chromosome of Pan paniscus (Fig.2.4), as well as in the homologous chromosome of man, gorilla and orangutan. The region which appears as an interstitial C-band most probably corresponds to a region which is present only in Pan troglodytes, and can be demonstrated by comparing the G-banded pattern of this chromosome with that of its homologues in all other species of Hominidae. Another difference between Pan troglodytes and Pan paniscus is that of chromosome No.23 which is metacentric in Pan paniscus and acrocentric in Pan troglodytes. The Y chromosome of the two species is different in size (compare Table 2.1 and 2.2), and in Pan paniscus it is a more metacentric chromosome than in Pan troglodytes. Both Y chromosomes are pale with quinacrine fluorescence. It is important to remark that these two species do not show secondary constrictions in any of their metacentric chromosomes, as is the case in man (chromosome 1, 9 and 16) or gorilla (chromosome 17 and 18). The regions of the centromere which are positively C-banded are variable in size but such variations/

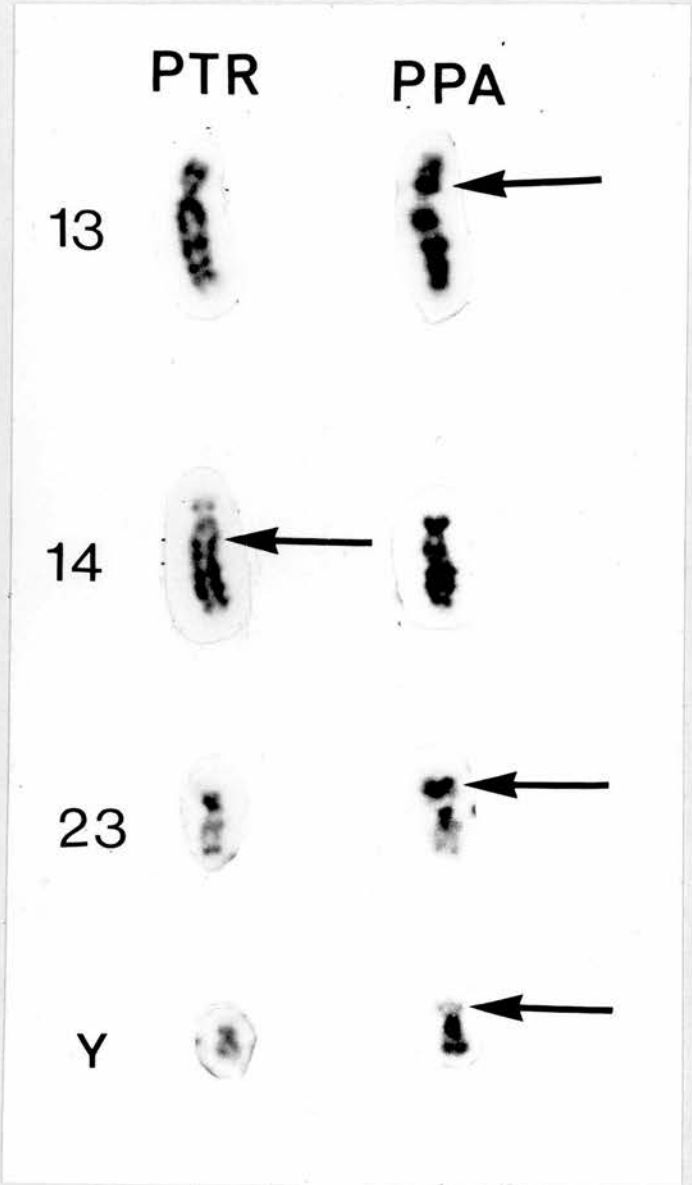


Fig.2.4. Differences between the chromosomes of Pan troglodytes (PTR) and Pan paniscus (PPA). Arrows point to G-band regions which are present in one species but absent from the other.

variations are minimal.

(D) Chromosome heteromorphisms in Pan troglodytes and Pan paniscus.

Study of the animals.

A group of 11 animals was studied, 8 of which were of the species Pan troglodytes and three of which were Pan paniscus. All animals were normal and healthy and were kept at two different centres: Bristol Zoo (England), and Yerkes Regional Primate Centre (Atlanta, USA). In Table 2.4 all animals of the adult generation are listed as generation I, and the offspring of these animals are listed as generation II. Animals of the adult generation are apparently not related and were caught wild. The only offspring (II.1) was born in captivity, and her parents are I.1 and I.2.

Chromosome analysis.

All animals had a normal chromosome constitution, but there was a clear amount of chromosome polymorphism within and between different individuals in the two species. The most obvious polymorphisms were those of the brilliant fluorescent regions of the autosomes (C-band regions showed less evident variations in size). Q-band polymorphisms were found in five pairs of autosomes. Such variations were in size: small (+), medium (++), or large (+++) (Fig.2.5), and intensity: brilliant (B), intense (I), medium (M), pale (P) or negative (N) (Paris Conference, 1971). The kinds of Q-band polymorphisms found are listed in Table 2.5.

(E) Discussion.

Our results show that both species of chimpanzee have a very similar/

TABLE 2.4

REGISTRY OF THE ANIMALS

Pan troglodytes

| | | | | |
|---|---|---|---|---|
| I.1 | BUTTONS | (Bristol) | II.1 EVE | (Bristol) |
| I.2 | ELIZABETH | (Bristol) | | |
| I.3 | SUSAN | (Bristol) | | |
| I.4 | JOHN | (Yerkes) | | |
| I.5 | HAL | (Yerkes) | | |
| I.6 | DOBBS | (Yerkes) | | |
| I.7 | JAMES | (Yerkes) | | |

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Pan paniscus

| | | |
|---|---|---|
| I.1 | BOSONDJO | (Yerkes) |
| I.2 | LOKALEMA | (Yerkes) |
| I.3 | MATATA | (Yerkes) |

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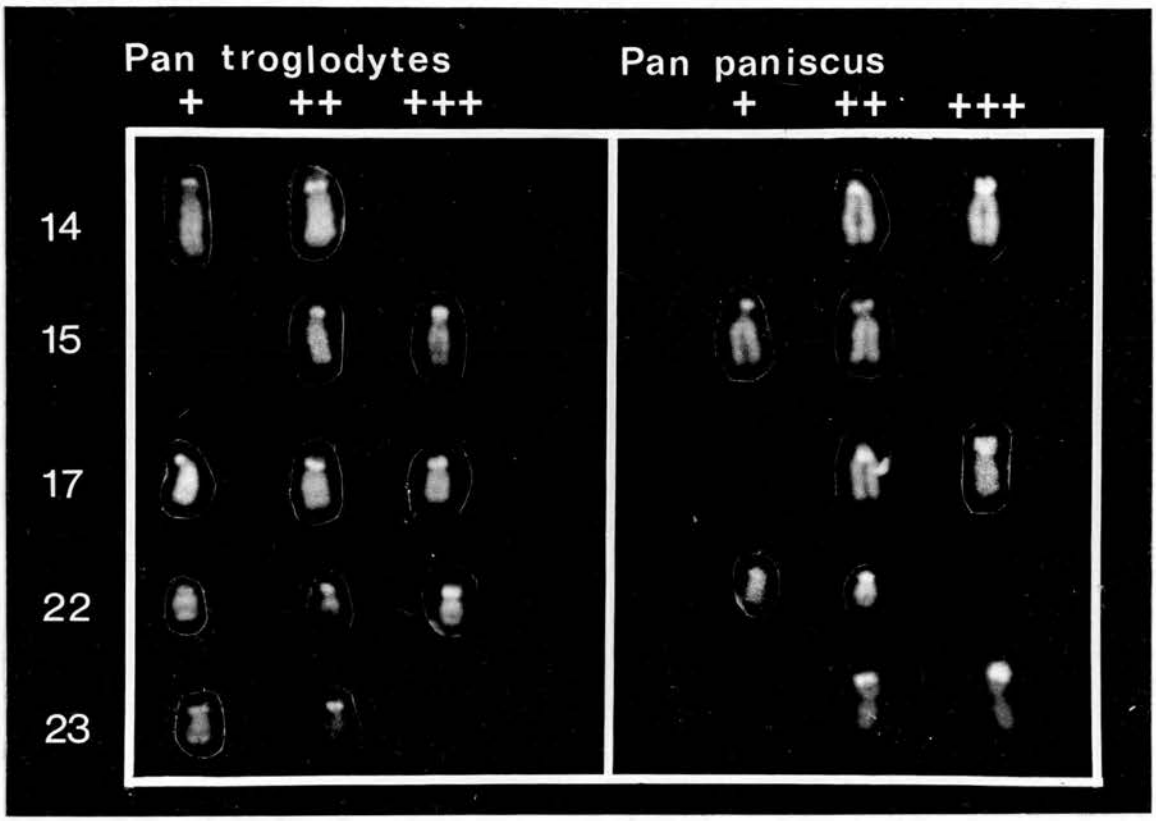


Fig.2.5. Size variations of brilliant fluorescent regions in Pan troglodytes and Pan paniscus.

TABLE 2.5

Q-BAND POLYMORPHISMS IN Pan troglodytes AND Pan paniscus

| Pan troglodytes Animal | Chromosomes 14 | 15 | 17 | 22 | 23 |
|---|---|---|---|---|---|
| I.1 | ++/++ | ++/+++ | +/++ | +/++ | ++/++ |
| I.2 | +/++ | I/++ | I/+ | ++/+++ | ++/++ |
| I.3 | +/++ | ++/++ | +++/+++ | I/+ | +/++ |
| I.4 | N/++ | ++/+++ | M/++ | I/++ | ++/++ |
| I.5 | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ |
| I.6 | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ |
| I.7 | ++/++ | ++/++ | ++/++ | ++/++ | +/+ |
| II.1 | ++/++ | I/++ | I/+ | +/++ | ++/++ |
| **Pan paniscus Animal** | | | | | |
| I.1 | ++/+++ | +/++ | ++/++ | ++/++ | ++/+++ |
| I.2 | ++/++ | ++/++ | +++/+++ | +/+ | ++/++ |
| I.3 | ++/++ | ++/++ | +++/+++ | +/+ | ++/+++ |

Brilliant ( +, ++, +++

I = Intense

M = Medium

P = Pale

N = Negative

similar chromosome constitution, since only three autosomes and the Y chromosome differ between species. Dutrillaux et al (1975b) has reported that chromosome 6 of Pan paniscus was different from chromosome 6 of Pan troglodytes in showing an extra R-band region at its long arm. However, with Q-, G- and R-banding we did not find any appreciable difference between this chromosome and its homologue in Pan troglodytes. The other differences between species are minimal since that of chromosome 13 only accounts for the short arm of this chromosome, that of chromosome 14 accounts for the absence of a C-band region in Pan paniscus, and that of chromosome 23 accounts for the existence of a larger region of positive C-banding and brilliant fluorescence in Pan paniscus. The differences between the Y chromosome are also minimal, and, with the exception of chromosome 13 in which the extra region in Pan paniscus was euchromatic, all others involved heterochromatic regions.

The regions where brilliant Q-band polymorphisms were found were the same as previously reported by other authors (Lejeune et al, 1973; Lin et al, 1973; Dutrillaux et al, 1975b) in the two species. The most extensive study of the brilliant fluorescent polymorphisms of Pan troglodytes is that of Lin et al (1973) who found that the regions of brilliant fluorescence were the proximal region of the short arm of all acrocentric chromosomes except chromosome 16. In Pan paniscus, chromosome 16 shows no brilliant fluorescence, and the region of brilliant fluorescence in the acrocentric chromosomes was also the proximal segment of the short arm. In one metacentric chromosome (No.23) brilliant fluorescence was located in the short arm well above the centromere. The number of regions which may show brilliant polymorphisms in both species of chimpanzee is lower than the number of/

of regions which may show brilliant polymorphisms in man. In man, brilliant polymorphisms may occur at chromosome 3, 4, 13, 14, 15, 21 and 22, and also in the Y chromosome. However, the estimated average number of brilliant polymorphisms in Pan troglodytes and Pan paniscus appears to be significantly higher than in man. In Pan troglodytes we have estimated that the average number of brilliant polymorphisms is 9.14 if the estimation is based on all animals except for animal II.1 which was related to I.1 and I.2. This estimation is based only on 7 animals, but a comparison with data in the literature confirmed that the average number of brilliant polymorphisms in Pan troglodytes was very high. Although it is difficult to compare data from different authors on Q-band polymorphisms since there are always variations due to the way these polymorphisms are scored by independent observers, a comparison is possible with the data reported by Lin et al (1973) based on 22 specimens of Pan troglodytes. In this report brilliant polymorphisms were classified as: (-) = little or no fluorescence; (+) = noticeable fluorescence; (++) = area of intense fluorescence almost twice the size of (+); and (+++) = most brilliant fluorescence, approximately three times the size of (+).

According to the illustration of these Q-band polymorphisms in the report of Lin et al (1973), it is evident that polymorphisms classified as (+), (++), and (+++) all represented brilliant regions of different size, whereas (-) represented regions where fluorescence was not brilliant. Thus, it was possible to estimate the average number of brilliant polymorphisms in Pan troglodytes which equalled 8.77. This frequency, though slightly below our own estimations is not significantly different since a contingency test gave a value of/

of $X_1^2 = 0.045$; $P > 0.90$. Pooling the data reported by Lin et al (1973) and ours, the average estimated incidence of brilliant polymorphisms in Pan troglodytes equals 8.85. Although this estimation is only based on 29 animals, it is significantly higher than the average number of brilliant and intense polymorphisms in human populations which range from 2.9 to 4.2 per individual (Buckton et al, 1976). A similar, though not identical situation is found in gorilla (see Chapter 3) in which the number of brilliant polymorphisms per animal is also very high compared to that of man. The chromosomes showing the highest incidence of brilliant polymorphism were Nos. 14 and 15 in our own estimations, which is in accordance with the data presented by Lin et al (1973). They reported the highest incidence in chromosome No.14.

In Pan paniscus all five pairs showed a brilliant fluorescent region in the three animals studied, thus the average number of brilliant polymorphisms per animal equalled 10. Other reports in the literature in which Q-banding has been reported in this species (Khudr et al, 1973; Dutrillaux et al, 1975b) are based on very few animals and this makes it impossible to make reliable estimations. However, Bogart and Benirschke (1977b) have reported Q-band polymorphisms in a family of Pan paniscus and found that the brilliant region of chromosome 23 was absent in one female animal who had transmitted this chromosome to five of her six offspring. However, the overall number of animals studied is still low to make any reliable estimation on the incidence of brilliant polymorphisms in this species.

(F) Summary

(1) The normal chromosome complements of Pan troglodytes and Pan paniscus have been compared and found to be very similar except for chromosome 13, 14, 23 and the Y chromosome.

(2) Chromosome polymorphisms have been found in both species in relation to the intensity and the size of quinacrine stained regions in chromosomes 14, 15, 17, 22 and 23.

(3) The average number of brilliant polymorphisms in Pan troglodytes has been estimated as 8.77, more than twice the average number of brilliant polymorphisms in human populations.

# CHAPTER 3: THE CHROMOSOMES OF THE GORILLA (Gorilla gorilla).

## (A) Introduction.

The Gorilla (Gorilla gorilla) is the great ape of greatest body weight and size. Wild populations of gorilla inhabit Africa in the western coast of the Cameroon and Gabon as well as in the high regions of eastern Zaire and Rwanda. Two races or subspecies of gorilla have been described; the one that inhabits the coast of Africa is commonly called the lowland gorilla (Gorilla gorilla gorilla), and the one that inhabits the high regions of central Africa is called the mountain gorilla (Gorilla gorilla berengei).

The chromosomes of both races of gorilla were first studied by Hamerton et al (1961, 1963) who found them both to have identical karyotypes. Detailed studies of the karyotype of this species have been reported with chromosome banding techniques (Dutrillaux et al, 1973; Miller et al 1974[a]). One of the most interesting findings in the gorilla is that the Y chromosome, like the human Y chromosome (but unlike the Y chromosome of all other mammalian species so far studied), has a brilliant fluorescent region at its long arm distal tip (Pearson et al, 1971). This feature is a useful tool for identification of the Y chromosome in chromosome preparations of gorilla, but its consistent identification in the spermatozoa has been so far unsuccessful (Seuanez et al, 1976d; see Chapter 9).

Detailed studies of the chromosome complement of the gorilla and on its presumptive homologies with other hominoids including man have been presented in the report of the Paris Conference (1971); supplement (1975). The recommendations of this report will be followed/

followed in this study, although some modifications will be proposed.

(B) The normal karyotype of Gorilla gorilla.

The gorilla has 48 chromosomes; 23 pairs of autosomes and the sex chromosomes. Of the autosomes, 15 pairs are metacentric, one pair is subtelocentric and 7 pairs are acrocentric (Fig. 3.1). The metacentric pairs comprise a group of large chromosomes (pairs 1 to 5), a group of medium sized chromosomes (pairs 6 to 10), and a group of small chromosomes (pairs 17 to 21). The acrocentric chromosomes comprise a group of large chromosomes (pairs 12 to 16), and a group of small chromosomes (pairs 22 and 23). All the acrocentric chromosomes in the species have a short arm a stalk and terminal satellite. They participate in satellite association, although pairs 22 and 23 are those most usually associated. The remaining autosome pair, No.11 is the only subtelocentric pair in the chromosome complement. Its short arm has no secondary constriction or satellite, and is never involved in association with other chromosomes. The X chromosome is a medium sized metacentric, and the Y chromosome is submetacentric. The fundamental number in this species can be considered as 79 if the subtelocentric chromosome and the Y chromosome (No.11) are counted as one armed in an individual with a 48, XY constitution. Detailed data on chromosome length and centromeric index are shown in Table 3.1.

Chromosome analysis with chromosome banding techniques.

The Paris Conference (1971); supplement (1975) does not show a C-band karyotype of the gorilla. In the present studies all cells have/

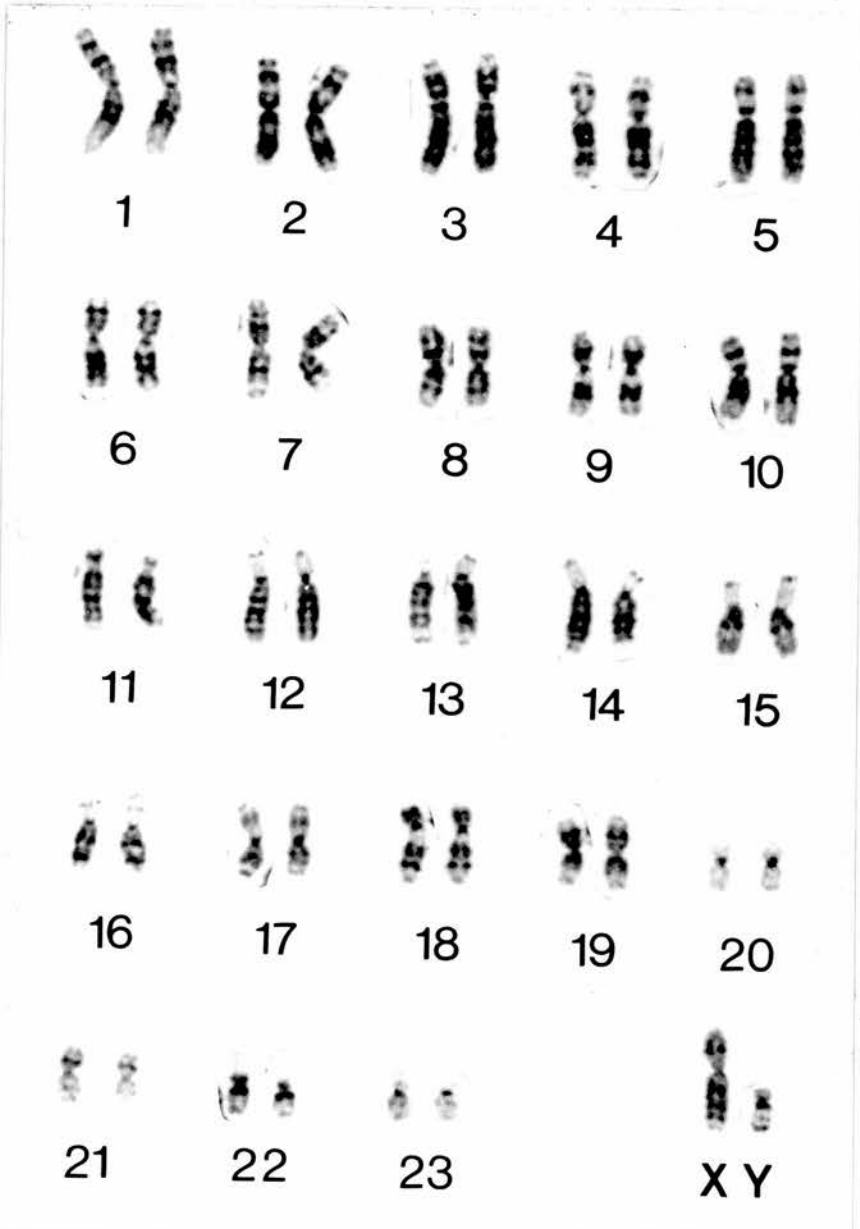


Fig.3.1. G-band karyotype of Gorilla gorilla.

Table 3.1

RELATIVE LENGTH OF GORILLA CHROMOSOMES

| CHROMOSOME NUMBER | CHROMOSOME LENGTH (%) | SHORT ARM LENGTH(%) | LONG ARM LENGTH(%) | CENTROMERIC INDEX |
|---|---|---|---|---|
| 1 | 7.73± 0.40 | 3.25 | 4.48 | 42.04 |
| 2 | 6.55± 0.33 | 2.90 | 3.65 | 44.27 |
| 3 | 6.15± 0.22 | 2.17 | 3.98 | 35.28 |
| 4 | 5.65± 0.19 | 2.10 | 3.55 | 37.16 |
| 5 | 5.68± 0.28 | 2.09 | 3.59 | 36.79 |
| 6 | 5.09± 0.19 | 1.77 | 3.32 | 34.77 |
| 7 | 4.66± 0.32 | 2.06 | 2.60 | 44.20 |
| 8 | 4.56± 0.22 | 2.04 | 2.52 | 44.73 |
| 9 | 4.38± 0.25 | 1.59 | 2.79 | 36.30 |
| 10 | 4.68± 0.26 | 1.88 | 2.80 | 40.17 |
| 11 | 4.42± 0.19 | 0.83 | 3.59 | 18.77 |
| 12 | 4.58± 0.26 | 0.78 | 3.80 | 17.03 |
| 13 | 4.44± 0.36 | 0.75 | 3.69 | 16.89 |
| 14 | 4.17± 0.35 | 0.74 | 3.43 | 17.74 |
| 15 | 3.71± 0.38 | 0.87 | 2.84 | 23.45 |
| 16 | 3.59± 0.22 | 1.01 | 2.58 | 28.13 |
| 17 | 3.74± 0.33 | 1.63 | 2.11 | 43.58 |
| 18 | 3.86± 0.30 | 1.02 | 2.84 | 26.42 |
| 19 | 3.43± 0.35 | 1.40 | 2.03 | 40.81 |
| 20 | 2.70± 0.20 | 1.04 | 1.66 | 38.51 |
| 21 | 2.51± 0.31 | 0.96 | 1.55 | 39.24 |
| 22 | 1.76± 0.21 | 0.42 | 1.34 | 23.86 |
| 23 | 1.73± 0.22 | 0.51 | 1.22 | 29.47 |
| X | 5.12± 0.30 | 1.77 | 3.35 | 34.17 |
| Y | 2.18± 0.31 | 0.59 | 1.59 | 27.06 |

Numbers represent percentages of haploid autosome complement.

have been Q-banded and then C-banded, and double karyotypes have been prepared as shown in Fig. 3.2. The following chromosomes require special comment.

Chromosome 11.

This chromosome is considered as homologous to the long arm of chromosome 2 in man, which is obvious with G- and with Q-banding (Fig.3.1 and Fig. 3.2). With C-banding this chromosome differs from all others in the complement by not having a heterochromatic region at the centromere.

Chromosome 12.

This chromosome is considered homologous to the short arm of chromosome 2 in man (Paris Conference 1971; supplement 1975). However, in the latter report GGO 12 is drawn diagramatically as a subtelocentric chromosome with a sold short arm. Our findings clearly show that with G- and Q-banding the short arm of this chromosome has three different regions: a proximal short arm region, a stalk and terminal satellite (Fig.3.1 and Fig.3.2).

Chromosome 18.

No homologous chromosome has been recognized in man (or in other Hominidae) for GGO 18 in the Paris Conference (1971); supplement (1975). However, we have proposed (see Mitchell et al, 1977) that this chromosome should be considered homologous to chromosome 9 in man (HSA 9). GGO 18 has a secondary constriction which, like the one of human chromosome 9, is located below the centromere. With C-banding, this secondary constriction stains as a dark block, and is/

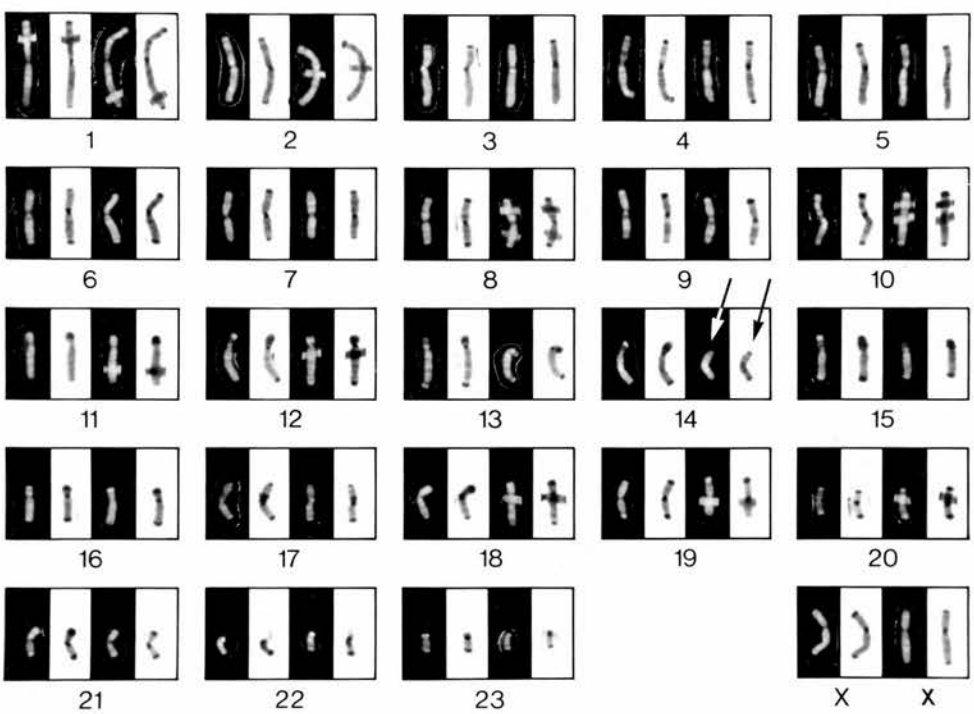


Fig.3.2 Q- and C-band karyotypes of Gorilla gorilla. Arrows point to variant chromosome 14.

is variable in size in a way comparable to the polymorphic sub-centromeric region of HSA 9. The G-banding patterns of GGO 18 and HSA 9, though not strictly identical, are remarkably similar (Fig.3.1 and Fig.3.2). GGO 18 shows two positive G-bands at the short arm, one above the centromere and one near the telomere. The long arm shows two positive G-bands which are distal to the region of the secondary constriction, a region which is negatively stained with Giemsa as well as with quinacrine. Detailed studies of the nature of DNA sequences in the region of the secondary constriction of GGO 18 have shown that four kinds of DNA sequences homologous to human satellite DNA I, II, III and IV (Gosden et al, 1977; Mitchell et al, 1977) are present. The situation is comparable to the secondary constriction region of HSA 9 which also contains satellite, I, II, III and IV (Gosden et al, 1975). It is because of this remarkable similarity in chromosome banding pattern and DNA content that we have postulated that GGO 18 should be considered as homologous to chromosome 9 in man.

The Y chromosome.

The Y chromosome of the gorilla is a submetacentric chromosome with a C.I. = 14.6 and a total length of approximately 2.5% of the haploid chromosome complement. When G-banded it stains mainly at the centromere (Fig.3.1), and with Q-banding it has a brilliant fluorescent region at the long arm distal region (Fig.3.3). The banding pattern of the Y chromosome of the gorilla and man are not, however, identical. The short arm of the Y chromosome in the gorilla shows medium intensity with fluorescence, and a pale centromeric region. The proximal region of the long arm also shows medium intensity/

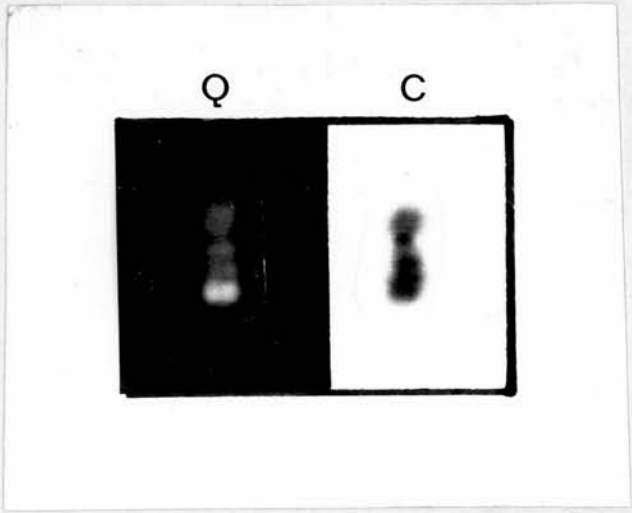


Fig.3.3 Q-and C-band patterns of the Y chromosome in Gorilla gorilla.

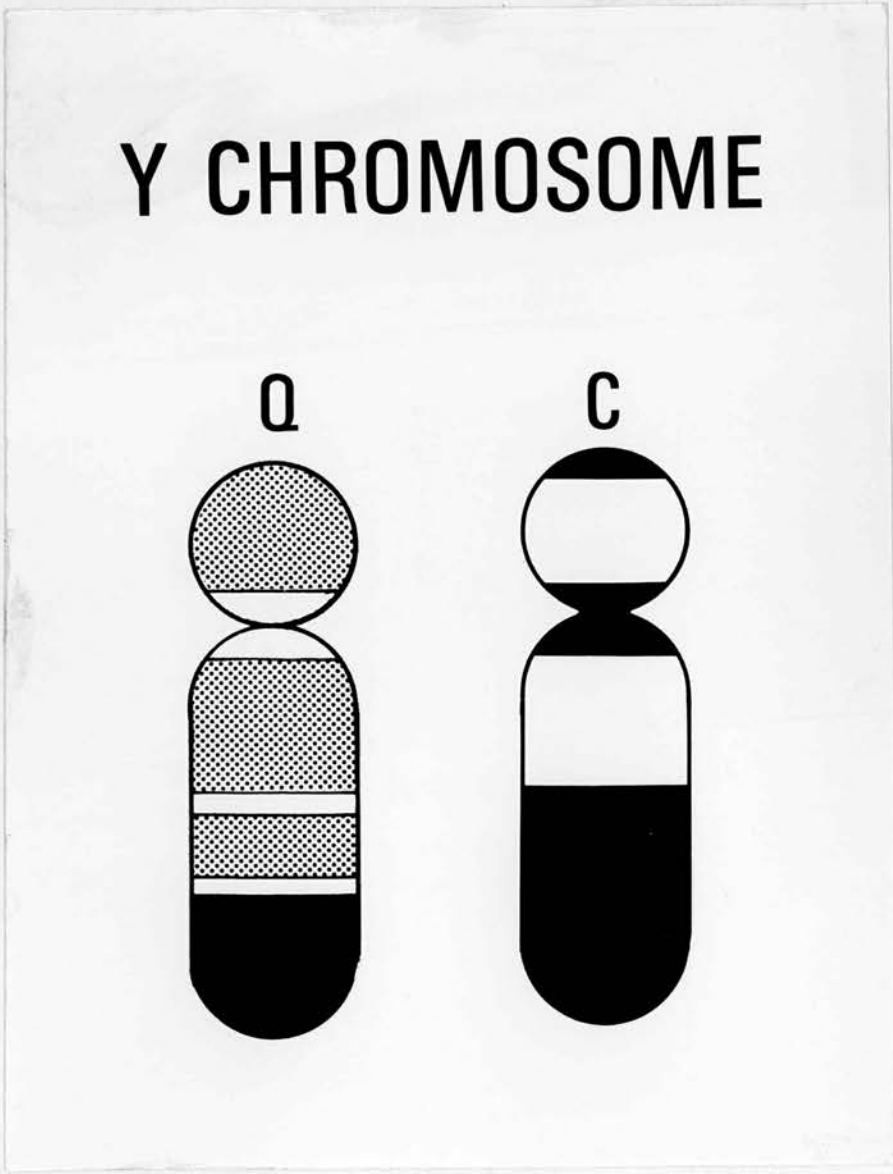


Fig.3.4. Diagram of the Q- and C-band patterns of the Y chromosome in Gorilla gorilla.

intensity down to the middle of the long arm where there is a narrow band of pale or negative fluorescence. The region of the long arm distal to that pale band comprises two regions: one, more proximal with medium intensity, the other more distal with brilliant fluoresce. C-banding on the same preparations show three different regions in this chromosome where heterochromatic segments are located. One is at the telomere of the short arm, a second is found at the centromere, and a third is at the long arm (Fig.3.3). The precise location of the Q- and C-banded regions is illustrated in Fig.3.4. The C-band region of the long arm covers the distal half of the long arm from the region of pale fluorescence to the telomere of the long arm. In the human Y chromosome, where there is no C-band at the short arm, the region which fluoresces brilliantly corresponds exactly to region of positive C-banding.

Terminal heteromatic regions in the gorilla.

The great majority of the chromosomes of the gorilla show terminal heterochromatic regions which are variable within animals and between animals. Some of these regions are defined as "variable regions" in the Paris Conference (1971); supplement (1975). The number of such regions seems to be, in our experience, higher than the number of regions listed in this report. This is probably the result of having observed a considerably larger number of animals (16 specimens) than in previous reports. Table 3.2 gives the list of terminal heterochromatic regions in the gorilla at each chromosome. These regions can be identified with quinacrine as terminal bands of medium intensity, and this can be noticed by comparing the Q- and C-banded karyotypes of the same cells. It appears to be that all metacentric/

TABLE 3.2

TERMINAL Q- AND C-BANDS IN Gorilla gorilla

| CHROMOSOME | p | q |
|---|---|---|
| 1 | + | + |
| 2 | + | + |
| 3 | + | - |
| 4 | + | + |
| 5 | + | + |
| 6 | + | + |
| 7 | + | + |
| 8 | + | + |
| 9 | + | - |
| 10 | + | + |
| 11 | + | + |
| 12 | (sat +) | + |
| 13 | (sat +) | + |
| 14 | (sat +) | + |
| 15 | (sat +) | + |
| 16 | (sat +) | + |
| 17 | + | + |
| 18 | + | + |
| 19 | + | + |
| 20 | + | + |
| 21 | + | + |
| 22 | (sat -) | + |
| 23 | (sat -) | + |
| X | - | - |

+ = positive banded region
- = negative banded region
p = short arm of chromosome
q = long arm of chromosome

metacentric chromosomes may have terminal heterochromatic regions at both arms, except for chromosomes 3 and 9 which have them only at their short arm. Chromosomes 5 can have heterochromatic regions at both arms, although in Fig. 3.2 only the short arm has a terminal band. In the dual karyotype of Fig. 3.8, the terminal band of the long arm of GGO 5 can be seen. All acrocentric chromosomes have a terminal band at their long arm, and the subtelocentric chromosome (No.11) shows a terminal region at both arms. The X chromosome shows no terminal band at its short arm, but the telomere of the long arm is occasionally faintly stained with Giemsa.

Brilliant fluorescent regions may be found on the following chromosomes in the gorilla: 3, 12, 13, 14, 15, 16, 22, 23 and the Y chromosome (Fig.3.2 and Fig.3.8). The regions where brilliant fluorescence is found are, however, not the same in all the autosomes. Apart from the fact that chromosome 3 shows brilliant fluorescence at the subcentromeric region, a region which is also C-banded positively, the brilliant fluorescent regions of the large acrocentrics differ from those of the small acrocentrics. In the large acrocentrics (Fig.3.2 and Fig.3.8) which comprise pairs No.12 to 16 the proximal region of the short arm shows usually medium fluorescence, but it has never been found to be brilliant. The satellite stalk is very pale or negatively stained, and only satellites are brilliant. In the small acrocentrics (pairs No.22 and 23), it is the proximal region of the short arm the one showing brilliant fluorescence. The satellite stalks are very pale or negatively stained as well as the satellites. The relationship between Q- and C-band regions is as follows: in the large acrocentrics (pairs 12 to 16) the proximal region of the short arm (showing medium intensity with quinacrine fluorescence/

fluorescence) is negatively C-banded whereas the satellite stalks and satellites are positively C-banded (Fig.3.5). This is well illustrated in a case where there has been a duplication of the satellite region in one large acrocentric (Fig.3.11) in which the sequence of Q- and C-banding of the short arm shows clearly the correspondence between regions within the short arm. In the small acrocentric chromosomes, however, it is the proximal region of the short arm that is positively C-banded, while the stalks and the satellites are negatively stained. This is clearly evident in Fig.3.6 in which three of these chromosomes are associated by their satellite regions.

(C) Chromosome heteromorphisms in Gorilla gorilla

Study of the animals.

A group of 16 animals, all of which were lowland gorillas (Gorilla gorilla gorilla) was studied. All animals were phenotypically normal and healthy, and were kept at two different centres: Briston Zoo (England), and Yerkes Primate Research Center (Atlanta, U.S.A.). All animals of the adult generation had been caught wild, and all animals of the young generation were born in captivity. Fig. 3.7 shows the pedigree of the animals and Table 3.3 shows their names and places of captivity.

Chromosome analysis.

All animals had a normal chromosome constitution, but the amount of chromosome polymorphism was very high. This was observed when comparing the chromosomes within as well as between individuals. A comparison/

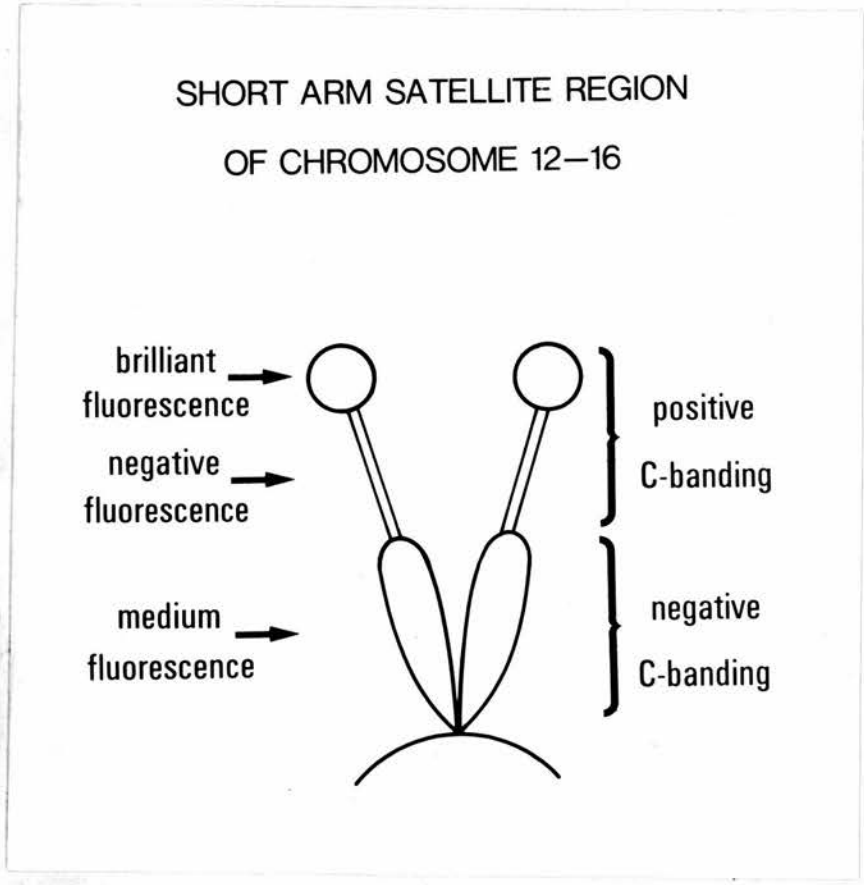


Fig.3.5. Diagram of the Q- and C-band short arm region of the large acrocentric chromosomes (Nos. 12 to 16) in Gorilla gorilla.

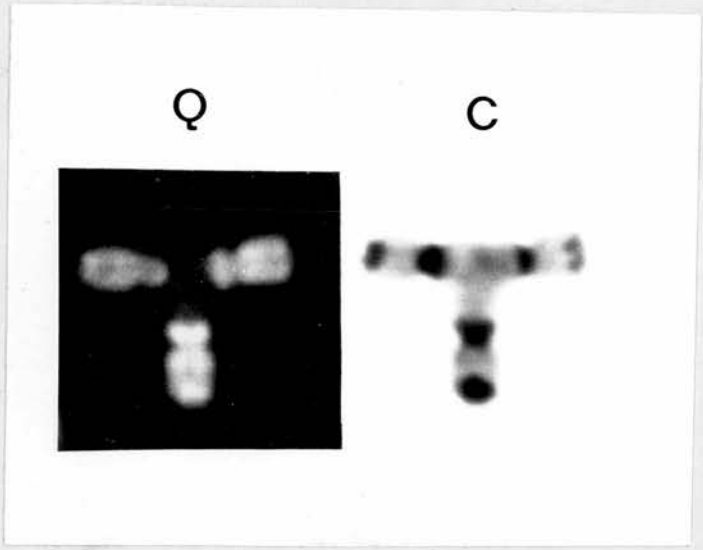


Fig.3.6. Q- and C-band patterns of the short arm region of the small acrocentric chromosomes (No.22 and 23) in Gorilla gorilla.

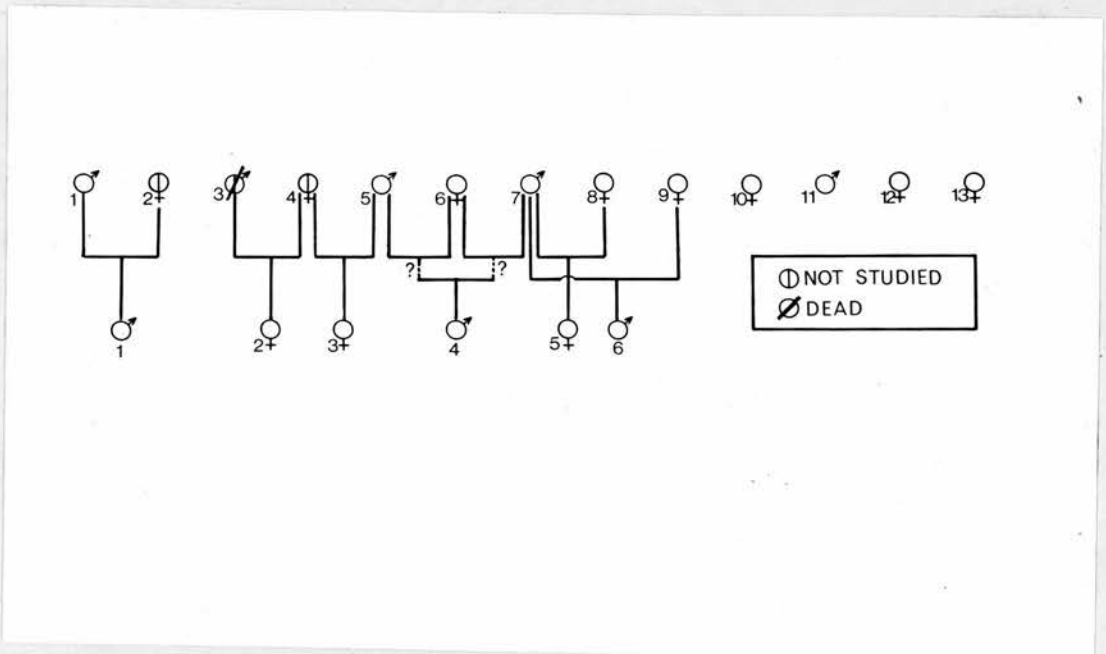


Fig.3.7. Gorilla pedigree. I.6 and I.7 are carriers of a deletion in chromosome 14. The deletion was not found in their offspring. II.4 is the offspring of the female I.6 and either I.5 or I.7.

TABLE 3.3

REGISTRY OF THE ANIMALS

| | | | | | |
|---|---|---|---|---|---|
| I. 1 | SAMPSON | (Bristol) | II.1 | DANIEL | (Bristol) |
| I. 2 | DALILAH | (Bristol) | II.2 | KISHINA | (Yerkes) |
| I. 3 | OZUM | (Yerkes) | II.3 | FANYA | (Yerkes) |
| I. 4 | PAKI | (Yerkes) | II.4 | AKBAR | (Yerkes) |
| I. 5 | CALABAR | (Yerkes) | II.5 | MAKI | (Yerkes) |
| I. 6 | SHAMBA | (Yerkes) | II.6 | BOMBOM | (Yerkes) |
| I. 7 | RANN | (Yerkes) | | | |
| I. 8 | CHOOMBA | (Yerkes) | | | |
| I. 9 | SEGOU | (Yerkes) | | | |
| I.10 | KATOOMBA | (Yerkes) | | | |
| I.11 | OBAN | (Yerkes) | | | |
| I.12 | BANGA | (Yerkes) | | | |
| I.13 | JINI | (Yerkes) | | | |

comparison within individuals showed that it was possible to distinguish between members of many chromosome pairs by differences in the size of or the intensity of fluorescent regions (Q-band polymorphisms), or in the size of constitutive heterochromatic regions (C-band polymorphisms). Differences between individuals were equally evident. Three animals showed chromosomes in which changes in the size of these regions had been so drastic that striking variations in chromosome morphology were produced. Two animals showed a deleted chromosome (No.14), and a third animal showed two metacentric chromosomes of unusual length and arm ratio (No.17 and No.19) resulting from an increase in length of the short arm. These three kinds of variant chromosome and the more common kinds of Q- and C-band polymorphisms will be described.

Variant chromosome 14.

A variant chromosome 14 was found in two animals (I.6 and I.7; Fig.3.7). It consisted of a deletion of the entire short arm satellited region, thus, del(14)(p13→p11:),(Fig.3.2). Both animals were phenotypically normal and of proven fertility. None of their offspring carried this variant chromosome.

Variant chromosome 17.

A heteromorphic pair 17 in which one chromosome was considerably larger in size than the other was found in one animal (I.1); Fig.3.8. With Q- and C-banding it was shown that this chromosome had a longer than normal secondary constriction region which was positively C-banded.

Variant/

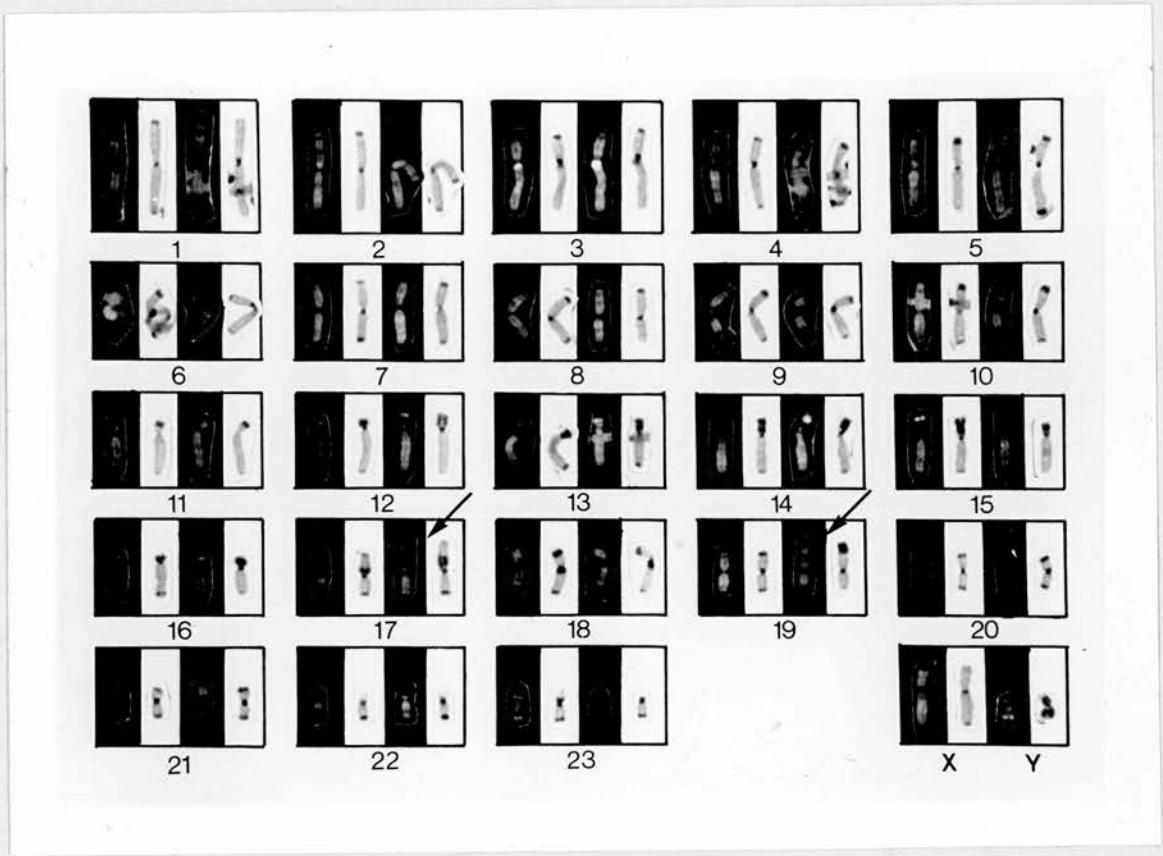


Fig.3.8. Q- and C-band karyotypes of animal I.1. Arrows point to variant chromosomes.

Variant chromosome 19.

A heteromorphic pair 19 was found in the same animal (I.1). The short arm of this chromosome was considerably longer than that of its normal homologue, and with Q- and C-banding it was again demonstrated that this increase in length was attributable to an increase in size of the terminal heterochromatic region on the short arm of this chromosome (Fig.3.8).

Q-band polymorphisms.

The following variations have been found:

(a) variation in the size of brilliant fluorescent regions which can be graded as small (+), normal (++) or large sized (+++)(Fig.3.9);

(b) variation in the intensity of fluorescence. The degree of intensity is that defined by the Paris Conference (1971): brilliant (B), intense (I), medium (M), pale (P), and negative (N).

No variations have been observed in the size or in the intensity of the distal region of the Y chromosome in seven male animals studied. Variations in the size of the terminal Q-band regions showing medium intensity were also detected, but these regions could be better demonstrated with C-banding techniques. The size of the negatively stained secondary constriction of chromosomes 17 and 18 was also found to be variable. These regions, as well as those of the satellite stalks in the chromosomes 12 to 16, correspond to regions of constitutive heterochromatin, and can better be demonstrated with C-banding techniques.

The fact that all animals have been studied with G-, Q- and C-banding techniques permitted to distinguish between situations in which/

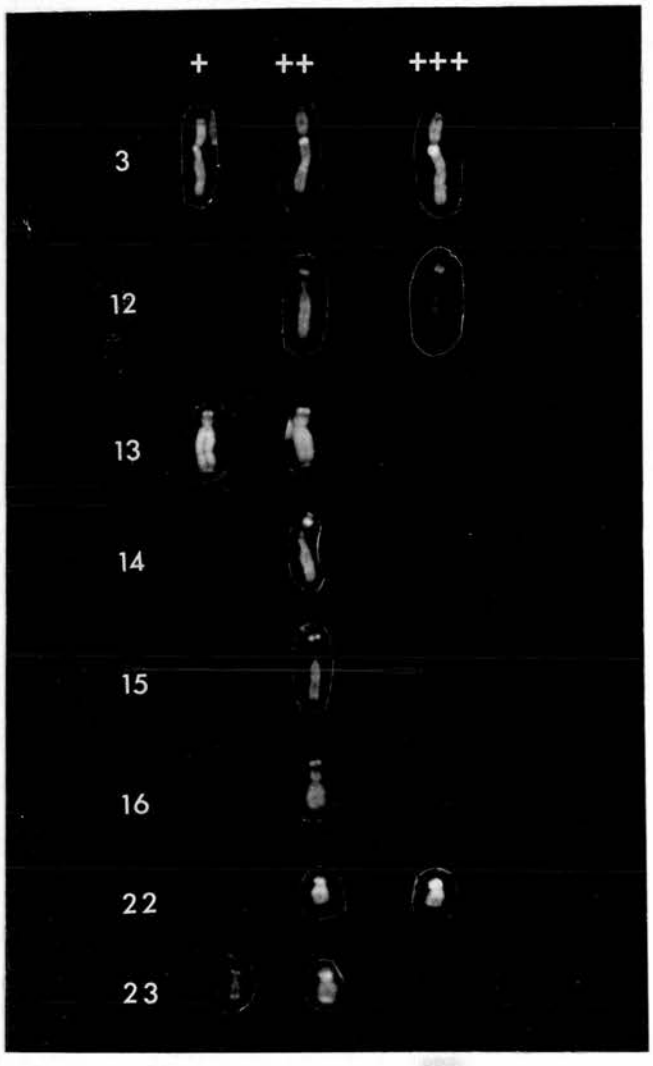


Fig.3.9. Variation in size of brilliant fluorescent autosomal regions in Gorilla gorilla.

which one region showed negative fluorescence as a consequence of not being present (e.g. the satellite region of chromosome 14 in del(14)(p13→p11:)) from regions which were present but showed pale or negative fluorescence. Data on Q-band polymorphisms are presented in Table 3.4. The frequency of brilliant fluorescent regions has been estimated from Table 3.4 and Fig.3.7; estimations are based on 10 animals (all animals of the adult generation) to avoid estimates on frequencies from related animals (e.g. father and son). The data are presented in Table 3.5 which also includes data from human populations (Mikelsaar et al, 1975; Buckton et al, 1976) for comparison.

C-band polymorphisms.

The following variations have been found:

(a) variation in size of the secondary constriction of chromosomes 17 and 18 which could be either small (+), normal (++) or large sized (+++);

(b) variation in the size of the terminal C-bands which correspond to regions of positive Q-banding (Fig.3.10);

(c) variation in size of the satellite stalks in the acrocentric chromosomes of the large acrocentric chromosomes (12 to 16). The size of the satellites was, however, less variable. Any increase in length of the short arm of these chromosomes was due to an increase in length in the stalks.

Table 3.6 shows the size of secondary constriction of chromosomes 17 and 18. Fig.3.10 shows the most relevant variations in size of the terminal heterochromatic regions and of the short arm of the acrocentric chromosomes. Among the large metacentric chromosomes the/

TABLE 3.4

Q-BAND POLYMORPHISMS IN Gorilla gorilla.

| Animal | Chromosomes | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 | 12 | 13 | 14 | 15 | 16 | 22 | 23 |
| I.1 | ++/+++ | +/++ | +/++ | N/++ | ++/++ | ++/++ | ++/++ | I/+ |
| I.5 | ++/++ | ++/++ | N/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ |
| I.6 | +/++ | ++/++ | M/M | N/++ | P/++ | ++/++ | ++/++ | I/++ |
| I.7 | ++/+++ | ++/++ | ++/++ | N/++ | ++/++ | ++/++ | ++/++ | +/+ |
| I.8 | ++/+++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/+++ | +/++ |
| I.9 | +/++ | ++/++ | +/+ | ++/++ | ++/++ | ++/++ | ++/+++ | ++/++ |
| I.10 | +/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | +/+ |
| I.11 | ++/+++ | ++/++ | +/+ | ++/++ | ++/++ | ++/++ | ++/+++ | +/+ |
| I.12 | ++/++ | ++/++ | I/++ | ++/++ | ++/++ | ++/++ | I/++ | ++/++ |
| I.13 | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | +/+ |
| II.1 | ++/+++ | ++/+++ | +/+ | ++/++ | ++/++ | ++/++ | M/++ | I/+ |
| II.2 | ++/++ | ++/++ | I/M | ++/++ | ++/++ | ++/++ | ++/++ | I/+ |
| II.3 | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | M/++ |
| II.4 | +/++ | ++/++ | M/++ | ++/++ | P/++ | ++/++ | ++/++ | I/++ |
| II.5 | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/++ | ++/+++ | +/++ |
| II.6 | ++/++ | ++/++ | +/++ | ++/++ | ++/++ | ++/++ | ++/++ | +/++ |

Brilliant ( + , ( ++ , ( +++

I = Intense

M = Medium

P = Pale

N = Negative

TABLE 3.5

FREQUENCY OF FLUORESCENCE POLYMORPHISMS IN GORILLA AND MAN

COMPARISON BETWEEN HOMOLOGS OF BOTH SPECIES

| | | | Mikelsaar et al 1975 | Buckton et al 1976 |
|---|---|---|---|---|
| GGO 2 | -- | HSA 3 | 0.650 | 0.640 |
| GGO 3 | 1.00 | HSA 4 | 0.278 | 0.40 |
| GGO 12p11 | -- | HSA 2p | -- | -- |
| GGO 12p13 | 1.00 | | | |
| GGO 13p11 | -- | (no homologue chromosome in man) | | |
| GGO 13p13 | 0.80 | | | |
| GGO 14p11 | -- | HSA 13p11 | 0.844 | 0.334 |
| GGO 14p13 | 0.85 | HSA 13p13 | 0.041 | 0.092 |
| (No homologue chromosome | | HSA 14p11 | -- | -- |
| in the gorilla) | | HSA 14p13 | 0.099 | 0.098 |
| GGO 15p11 | -- | HSA 15p11 | -- | -- |
| GGO 15p13 | 0.95 | HSA 15p13 | 0.004 | 0.119 |
| GGO 16p11 | -- | HSA 18 | -- | -- |
| GGO 16p13 | 1.00 | | | |
| GGO 22p11 | 0.95 | HSA 21p11 | -- | 0.006 |
| GGO 22p13 | -- | HSA 21p13 | 0.080 | 0.096 |
| GGO 23p11 | 0.90 | HSA 22p11 | 0.359 | 0.024 |
| GGO 23p13 | -- | HSA 22p13 | 0.080 | 0.140 |

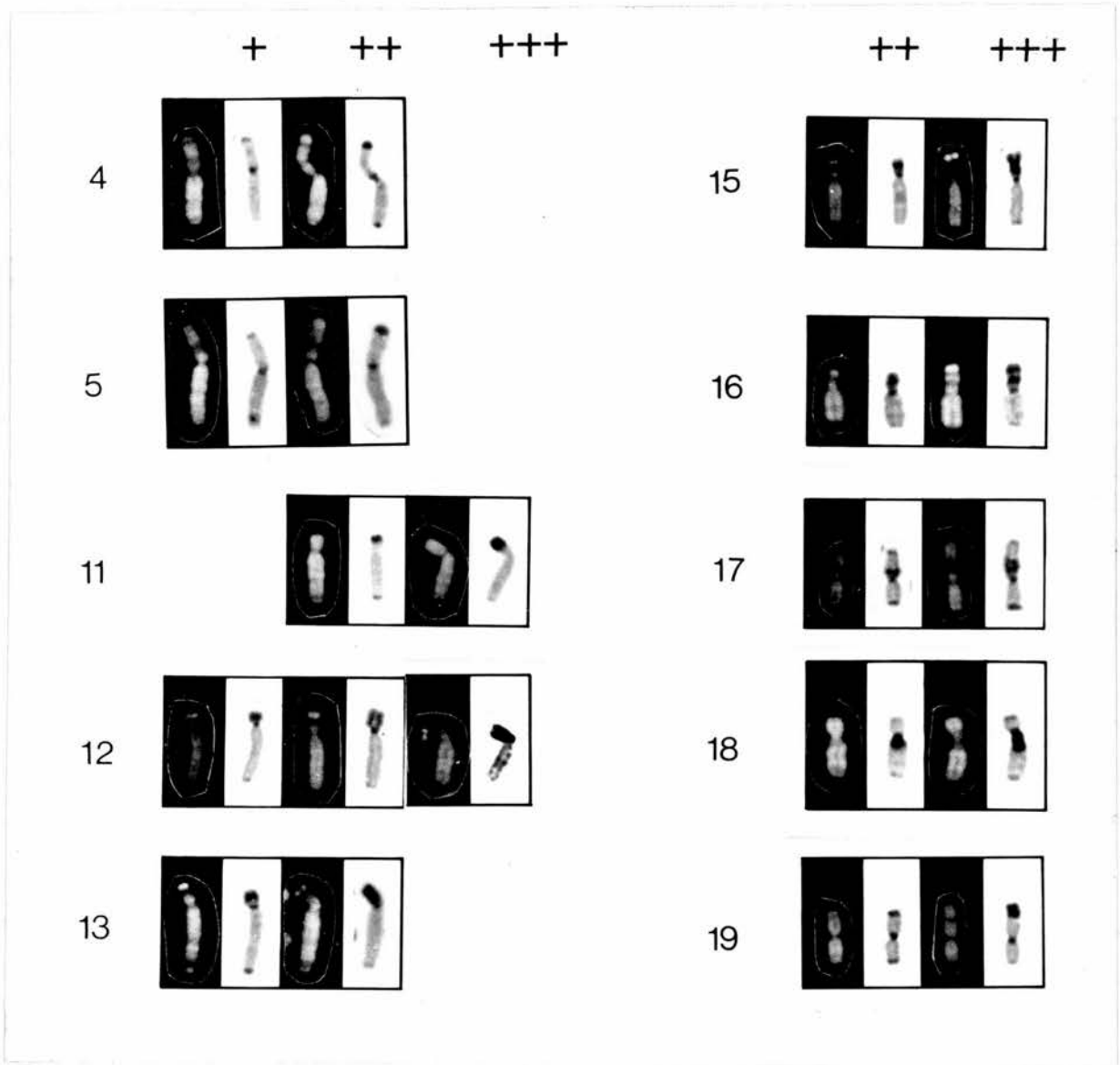


Fig.3.10. C-band polymorphisms in Gorilla gorilla. Chromosomes 4, 5, 11 and 19 show variation in the size of the C-band terminal region of their short arm. Chromosomes 12, 13 and 15 show variation in the size of the satellite stalk. Chromosome 16 shows a duplication of either the proximal short arm region or the terminal satellite (see Fig.3.11). Chromosomes 17 and 18 show variable secondary constriction regions. All chromosomes have been previously identified with Q-banding.

TABLE 3.6

C-BAND POLYMORPHISMS IN Gorilla Gorilla

(SECONDARY CONSTRICTION OF CHROMOSOMES 17 AND 18)

| Animal | Chromosome | |
|---|---|---|
| | 17 | 18 |
| I.1 | ++/+++ | ++/++ |
| I.5 | ++/++ | ++/++ |
| I.6 | ++/+++ | ++/++ |
| I.7 | ++/++ | ++/++ |
| I.8 | ++/+++ | ++/++ |
| I.9 | ++/+++ | ++/++ |
| I.10 | ++/++ | ++/++ |
| I.11 | ++/++ | ++/+++ |
| I.12 | ++/++ | ++/++ |
| I.13 | ++/++ | ++/+++ |
| II.1 | ++/++ | ++/++ |
| II.2 | ++/++ | ++/++ |
| II.3 | ++/++ | ++/++ |
| II.4 | ++/+++ | ++/++ |
| II.5 | ++/++ | ++/++ |
| II.6 | ++/++ | ++/++ |

the terminal region of the short arms of chromosomes 4 and 5 were most commonly found to vary. The short arm of the small metacentric (No.19) was frequently found to be variable. Among the large acricentric chromosomes, it was No.12 that showed greatest variation; the satellite stalks of this chromosome appeared sometimes twice as long as those of its normal homologue (Fig.3.10). A somewhat different situation was found in chromosome 16 in two animals (I.10 and I.11). Here, the satellite (or alternatively the proximal region of the short arm) had been duplicated. The comparison between the Q- and the C-banding patterns of the short arm of this chromosome is shown in Fig.3.10 and explained in the corresponding diagram (Fig.3.11).

(D) Discussion.

The variant chromosomes found in three animals had produced no visible phenotypic effect. Deletion of the short arm of chromosome 14 in the gorilla has been described previously (Miller et al, 1974a) and this chromosome was designated as No.17 in their classification. Although we consider GGO 14 as homologous to chromosome 13 in man following Paris Conference (1971); supplement (1975), the deleted region is not exactly similar to that of the corresponding human homologue. In the human homologue (No.13) the region P13→p11 is a site of hybridisation with cRNA complementary to the 18S and 28S cistrons, whereas in the gorilla these highly repeated sequences are located only at the short arms of chromosome 22 and 23 (Henderson et al, 1976a). Both in GGO 14 and HSA 13 there are satellite DNAs at the short arm; those detected in GGO 14 are homologous to human satellite I, II, III and IV (Gosden et al, 1977; Mitchell et al, 1977).

The/

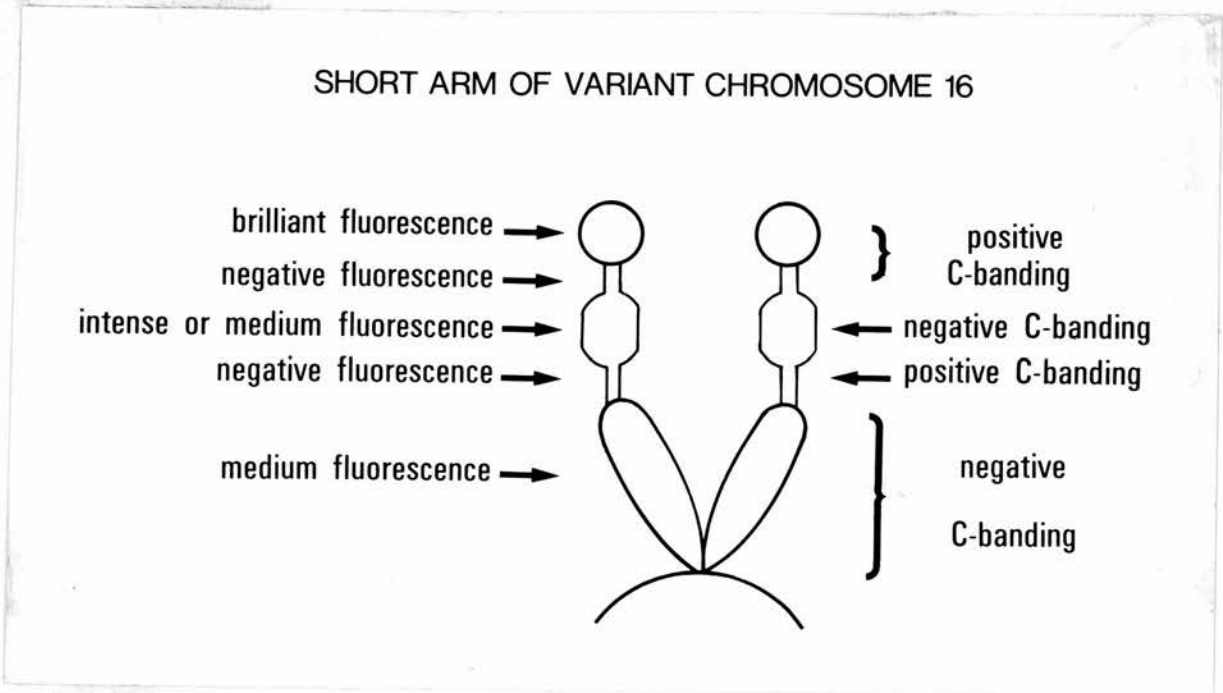


Fig.3.11. Diagram of the Q- and C-band short arm region of the variant chromosome 16.

The structural heteromorphisms observed in chromosome 17 and 19 were due to increases in the amount of constitutive heterochromatin, regions which most probably have low genetic activity. The secondary constriction region of chromosome 17 contains sequences that are homologous to the 4 human satellite DNAs (Gosden et al, 1977; Mitchell et al, 1977), which up to the present time are considered to be devoid of transcriptional activity.

Q-band polymorphisms are interesting since they show important differences between the gorilla and man. In the first place, the number of chromosomes which may show brilliant fluorescence is higher in the gorilla than in man (8 pairs of autosomes against 7 pairs in man). But, the most striking difference seems to be in the number of chromosomes that actually carry a brilliant fluorescent region in the gorilla compared to man. In our small group of 10 unrelated animals we have estimated that the average number of brilliant polymorphisms is 14.9 (autosomal polymorphisms), whereas population studies in man have shown that the average number ranges from 2.9 to 4.2 (Buckton et al, 1976). The regions where brilliant fluorescent chromatin is found are, however, not necessarily identical when chromosomes of similar morphology are compared between the two species (e.g. HSA 21 and GGO 22).

C-band polymoprhisms are also very common in the gorilla, and the surprisingly high number of variable regions is probably a result of the large amount of constitutive heterochromatin in the chromosome complement of this species. The explanation why so much constitutive heterochromatin is found in the gorilla is intriguing. We know that total DNA content is higher in the gorilla than in man and other hominoids (see Chapter 10), and that the gorilla has apparently a larger/

larger amount of highly repeated DNA sequences homologous to the four human satellite DNAs (see Chapter 6). Whatever the mechanism by which constitutive heterochromatin has appeared, its effect seems to be to make the chromosome complement of this species extremely variable.

(E) Summary - important points of this chapter.

(1) The normal chromosome complement of Gorilla gorilla gorilla has been studied with G- Q- and C-banding techniques. The following findings have provided new information which was not given in the Paris Conference (1971); supplement (1975):

(a) Chromosome No.11 (the homologue to the long arm of chromosome 2 in man) shows no C-band at its centromere region.

(b) Chromosome No.12 (the homologue to the short arm of chromosome 2 in man) has been found to be an acrocentric chromosome with a short arm, a satellite stalk and a terminal satellite.

(c) Chromosome No.18 has been considered to be homologous to chromosome No.9 in man.

(d) The Q- and C-banding patterns of the Y chromosome has been described in detail.

(e) The distribution of constitutive heterochromatin in the chromosome complement of the gorilla has been demonstrated.

(2) Q- and C-band polymorphisms have been studied in a group of 16 animals, all of which had a normal chromosome complement. Three variant chromosome types (N 14, 17 and 19) had resulted from drastic changes in the amount of constitutive heterochromatin. Q-band polymorphisms showed a high incidence of brilliant regions in this species (14.9 compared with approximately 3 in man). Brilliant polymorphisms were found in 8 pairs of autosomes, but no variation was found in the size or in the intensity of fluorescence in the Y chromosome. With C-banding, the secondary constriction regions of chromosome 17 and 18 were found to be variable in size, as well as many terminal heterochromatic regions/

regions of many autosome pairs, and the stalks of the satellites of the acrocentric chromosomes.

# CHAPTER 4: THE CHROMOSOMES OF THE ORANGUTAN (Pongo pygmaeus)

## (A) Introduction:

The orangutan (Pongo pygmaeus) is the only species of great ape which inhabits Asia. Wild populations of orangutan are found in the islands of Borneo and Sumatra, and two subspecies or races have been described; the Bornean race, Pongo pygmaeus pygmaeus, and the Sumatran race, Pongo pygmaeus abelii. Only minor phenotypic differences occur between the races (Jones, 1969; Eckhard, 1975), and hybrid animals can be produced if specimens of different race are allowed to mate in captivity. The chromosomes of the orangutan were first described by Chiarelli (1961), and later compared with those of man and the other great apes. When chromosome banding techniques became available, detailed studies of the chromosomes of the orangutan showed that there was no brilliant fluorescence as had been found for man and for the African apes (Pearson et al, 1971). It was also found that another family of the superfamily Hominoidea, the Hylobatidae (gibbons and siamangs) did not show brilliant fluorescence, and this finding gave further evidence in favour of the generally held view that man is more closely related to the African apes than to the Ponginae and the Hylobatidae (Pearson, 1973). A detailed description of the standardized karyotype of the orangutan has been presented in the report of the Paris Conference (1971); supplement (1975), and it will be followed in this study.

## (B) The normal chromosome complement of Pongo pygmaeus.

Chromosome/

Chromosome number and morphology.

The orangutan has a diploid number of 48 chromosomes, 23 pairs of autosomes and the sex chromosomes. Of the autosomes, 13 pairs are metacentric, one pair is subtelocentric, and 9 pairs are acrocentric (Fig. 4.1). The metacentric chromosomes comprise a group of large chromosomes (pairs 1 to 5), medium sized chromosomes (pairs 6 to 9), and small chromosomes (pairs 18 to 21). The acrocentric chromosomes comprise two groups, one of larger chromosomes (pairs 11 to 17) and another of small chromosomes (pairs 22 and 23). The acrocentric chromosomes in this species have all short arm-satellited regions and they are involved in satellite association. Chromosome No.10 is the only subtelocentric autosome in this species; it is a medium sized chromosome. The X chromosome is also medium sized, its length being almost equal to that of chromosome 6, although the former is a more metacentric chromosome. The Y chromosome is subtelocentric and is slightly larger than chromosomes 22 and 23. Detailed information of chromosome lengths is given in Table 4.1. (NF = 75 if No.10 and the Y chromosome are counted as one armed.)

Chromosome banding patterns

Detailed descriptions of the chromosome banding patterns of the orangutan have been given in the report of the Paris Conference (1971); supplement (1975). These recommendations of chromosome nomenclature given in the report have been followed (Fig. 4.1), and detailed descriptions of each chromosome will therefore not be reported in this study. Only those chromosomes which, in our opinion, have not been described in sufficient detail, or have been found/

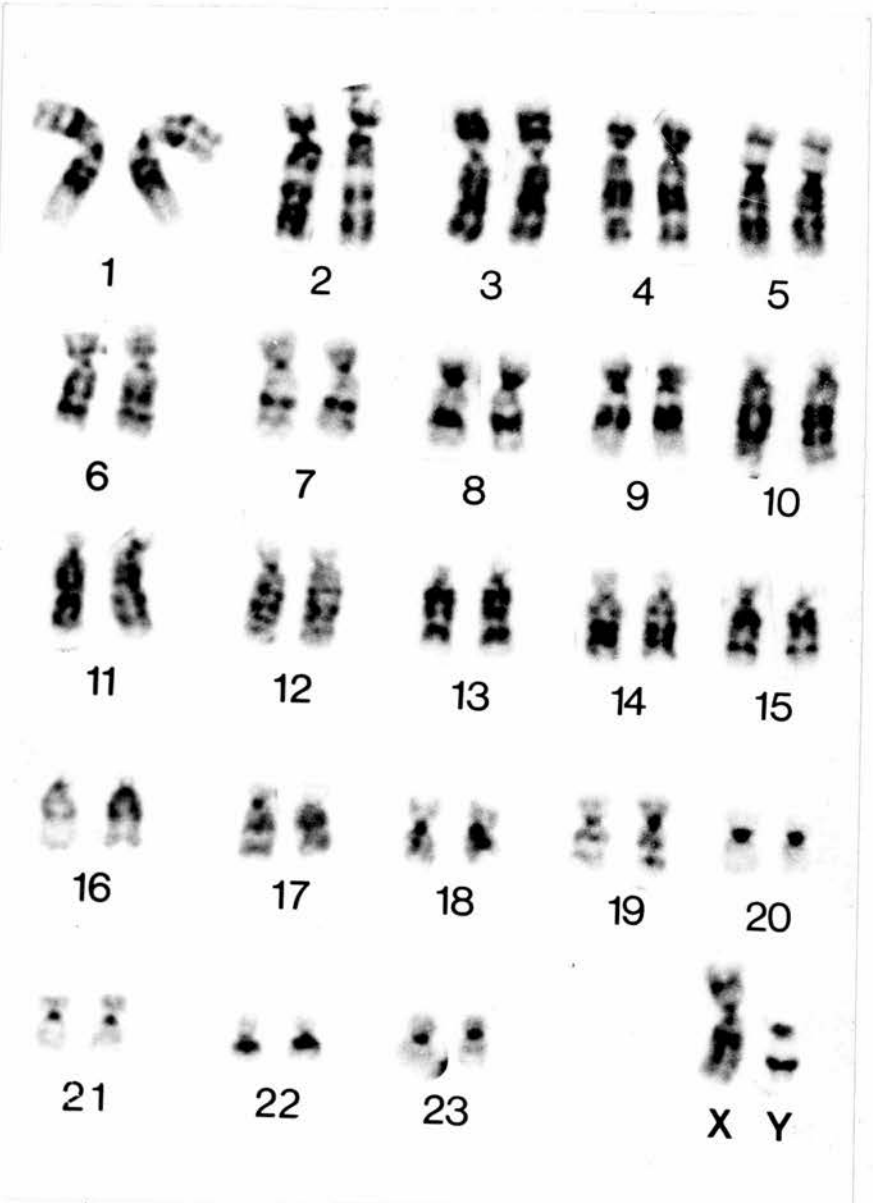


Fig.4.1. G-band karyotype of Pongo pygmaeus.

Table 4.1

RELATIVE LENGTH OF ORANGUTAN CHROMOSOMES

| CHROMOSOME NUMBER | CHROMOSOME LENGTH(%) | SHORT ARM LENGTH(%) | LONG ARM LENGTH(%) | CENTROMERIC INDEX |
|---|---|---|---|---|
| 1 | 8.33± 0.76 | 3.64 | 4.69 | 43.7 |
| 2* | 7.29± 0.29 | 1.38 | 5.91 | 18.9 |
| 3 | 6.42± 0.51 | 1.95 | 4.47 | 30.4 |
| 4 | 6.11± 0.35 | 1.58 | 4.53 | 25.9 |
| 5 | 5.78± 0.40 | 2.00 | 3.78 | 34.6 |
| 6 | 5.25± 0.39 | 1.64 | 3.61 | 31.2 |
| 7 | 4.44± 0.29 | 1.21 | 3.23 | 27.3 |
| 8 | 4.37± 0.32 | 1.28 | 3.09 | 29.3 |
| 9** | 4.43± 0.30 | 0.85 | 3.58 | 19.2 |
| 10 | 5.00± 0.58 | 0.58 | 4.42 | 11.6 |
| 11 | 4.74± 0.14 | 0.51 | 4.23 | 10.8 |
| 12 | 4.45± 0.27 | 0.58 | 3.87 | 13.0 |
| 13 | 4.12± 0.40 | 0.62 | 3.50 | 15.0 |
| 14 | 3.98± 0.18 | 0.50 | 3.48 | 12.6 |
| 15 | 3.47± 0.23 | 0.47 | 3.00 | 13.5 |
| 16 | 3.49± 0.34 | 0.36 | 3.13 | 10.3 |
| 17 | 3.11± 0.28 | 0.38 | 2.73 | 12.2 |
| 18 | 2.96± 0.22 | 1.17 | 1.79 | 39.5 |
| 19 | 3.12± 0.17 | 0.93 | 2.20 | 29.7 |
| 20 | 2.69± 0.20 | 1.12 | 1.57 | 41.6 |
| 21 | 2.21± 0.16 | 0.83 | 1.38 | 37.6 |
| 22 | 1.86± 0.10 | 0.51 | 1.35 | 27.4 |
| 23 | 1.99± 0.10 | 0.54 | 1.45 | 27.1 |
| X | 5.29± 0.31 | 1.97 | 3.32 | 37.2 |
| Y | 2.47± 0.33 | 0.36 | 3.11 | 14.6 |

Numbers represent percentages of haploid autosome complement.

* The variant (Bornean) chromosome 2 has a C.I. = 10.2

** The variant chromosome 9 has a C.I. = 34.7

found to show a different morphology and banding pattern will be described.

Chromosome 2.

For example, we have found that both the morphology and banding patterns of chromosome 2 (PPY 2) differs from those proposed at the report. PPY 2 is the second largest chromosome in the species, and it is clearly a submetacentric chromosome (Fig. 4.1). In the Paris Conference report (1971); supplement (1975), page 17, however, there is a photograph of the R-, Q-, and G-band patterns of the orangutan in which three No.2 chromosomes, one R-banded, one Q-banded and one G-banded have been assembled. The picture was composed obviously from three different cells, and probably from different animals, since this assemblage was made by combining data from different investigators. Fig. 4.2 illustrates how PPY 2 is shown in the Paris Conference (1971); supplement (1975), and it is evident that the morphology and banding pattern of the G-banded chromosome on the right does not correspond to the morphology and the banding patterns of the R- and Q-banded chromosomes on the left and centre. This slight discrepancy obviously went unnoticed when the picture was composed, and when the diagramatic version of the G-banded chromosome was prepared. In fact, this latter chromosome is a variant chromosome 2 which was described by Turleau et al (1975) in an individual family of orangutans. The normal chromosome 2, which by definition should be the one most commonly found in the species is represented accurately by the chromosome of the left (R-banded) and in the centre (Q-banded). The G-band pattern of the normal chromosome 2 is shown in the lower half of Fig. 4.2. The most/

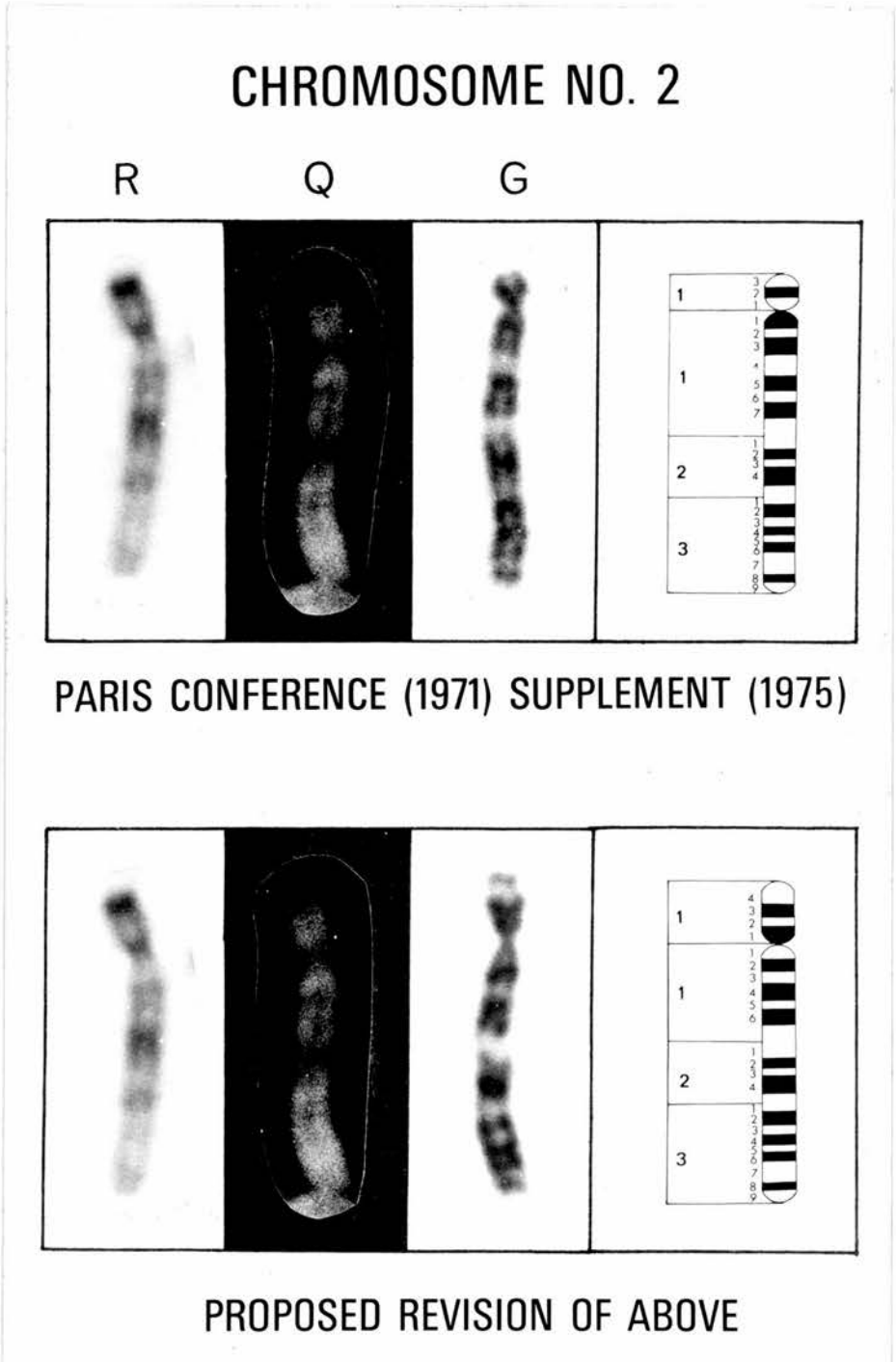


Fig.4.2. Chromosome No.2 in Pongo pygmaeus as illustrated in the Paris Conference (1971); supplement (1975), and our proposed revision.

most important difference to the one we propose as the normal PPY 2 and the one of the Paris Conference (1971); supplement (1975) is that the former has two positive G-bands at the short arm (and not one), but only one positive G-band below the centromere (and not two). The G-banded chromosome of the Paris Conference (1971); supplement (1975) will be initially considered as a variant chromosome 2, since it has appeared with a lower frequency than the other chromosome 2 in the species.

Chromosome 9.

No precise information on the C-banding patterns of the great apes is given in the Paris Conference (1971); supplement (1975). In the orangutan, all chromosomes show C-bands at their centrometric region except for one autosomal pair. PPY 9 has a similar morphology and Q- G- and R-banding patterns to chromosome 12 in man, but unlike man it does not have a heterochromatic region at its centromere (Fig. 4.3).

Y Chromosome.

The Y chromosome of the great apes is not described in detail in the Paris Conference (1971); supplement (1975) essentially because most of the studies were made on female animals. In our own studies, we have found that with G-banding, (Figs. 4.1, 4.9, 4.10 and 4.12), PPY Y is pale at its short arm, darkly stained at the centromere and negatively stained in almost all the long arm, except for a subterminal intense band. The telomere of the long arm is occasionally stained faintly with Giemsa. With Q-banding, this chromosome shows intense fluorescence, but it is not as brilliant as the long arm distal/

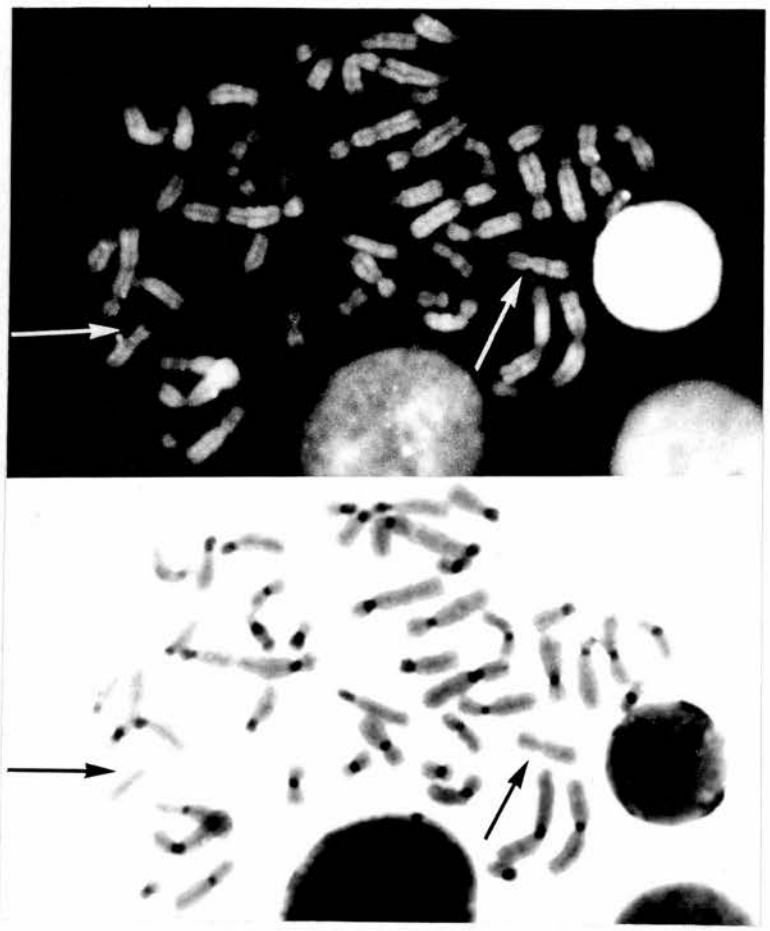

Fig.4.3. Q- and C-band patterns of Pongo pygmaeus. Arrows point to a chromosome pair (No.9) which shows no C-band region at the centromere. The pair arrowed is heteromorphic (see Figs. 4.7. and 4.8.).

distal segment of man and the gorilla. The short arm is intense, the centromeric and subcentromeric regions appear pale, and the rest of the long arm is uniformly intense except for its most distal region which is pale. C-banding on the same preparations (Fig. 4.4) shows two clear blocks of constitutive heterochromatin; a larger block at the centromeric-subcentromeric region, and another, smaller block at the distal end of the long arm. The regions of positive C-banding correspond to regions of pale fluorescence, those of intense fluorescence to negatively C-banded regions.

(C) Chromosome Heteromorphisms in Pongo pygmaeus.

Data on the animals.

A total number of 27 animals was karyotyped and studied. All animals were phenotypically normal, and in terms of origin, may be considered to fall into three different groups: Bornean (B), Sumatran (S) and hybrid individuals of the two populations (H) (Fig. 4.5). The origin and names of all animals are listed in Table 4.2 as well as their location in captivity. All animals of the adult generation (B.I. and S.I.) were caught wild in Borneo or Sumatra, and all those of the younger generation (B.II., S.II., and H.I.) were born in captivity. All adult animals were of proven fertility except for B.I.3 and B.I.4 which were mated recently for the first time, and S.I.16 which had never mated. However S.I.16 and S.I.17 had been electroejaculated, and sperm morphology was found to be normal in both animals (Martin et al, 1975; section II of this thesis). The frequency of normal sized regular shaped spermatozoa was above 98% in both animals. Two hybrid animals (H.I.1 and H.I.3) resulted from /

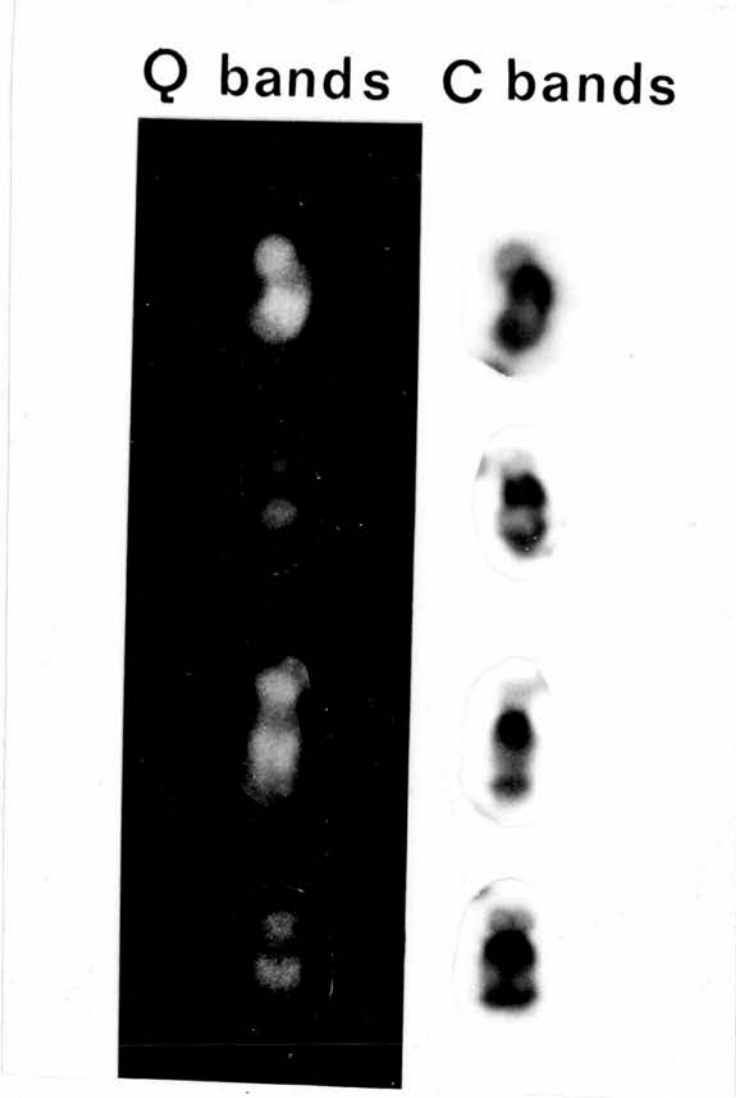


Fig.4.4. Q- and C-banding patterns of the Y chromosome in Pongo pygmaeus.

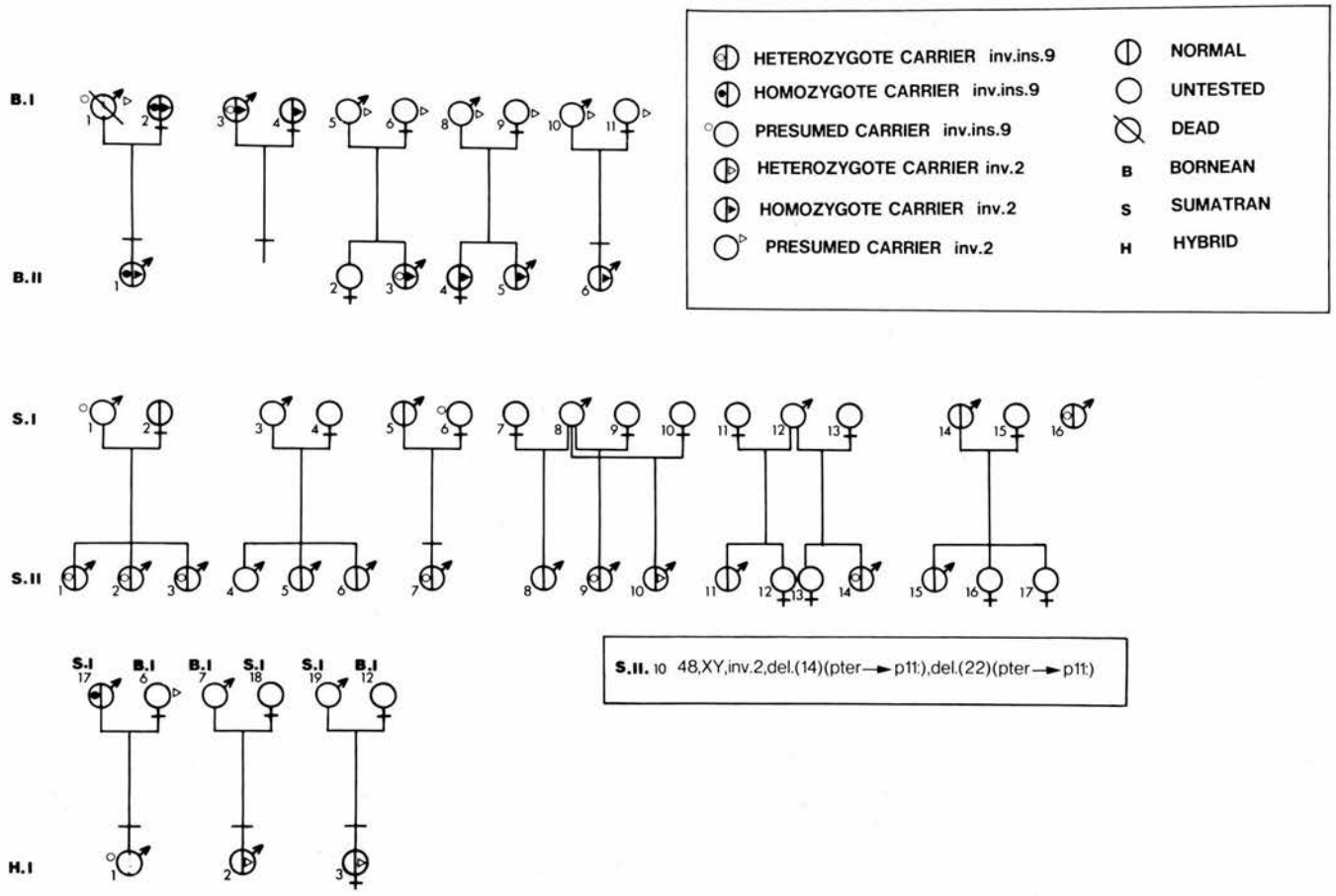


Fig.4.5. Orangutan pedigree.

TABLE 4.2

REGISTRY OF THE ANIMALS

BORNEAN

| | | | | | |
|---|---|---|---|---|---|
| B.I. 1 | JACK | (Bristol) | B.II. 1 | JAMES | (Bristol) |
| B.I. 2 | JILL | (Bristol) | B.II. 2 | POPI | (Yerkes) |
| B.I. 3 | ABANG | (Bristol) | B.II. 3 | TERIANG | (Yerkes) |
| B.I. 4 | DYANG | (Bristol) | B.II. 4 | SUKA | (London) |
| B.I. 5 | PADANG | (Yerkes) | B.II. 5 | JANTAN | (London) |
| B.I. 6 | PADDI | (Yerkes) | B.II. 6 | ANAK | (London) |
| B.I. 7 | BOY | (London) | | | |
| B.I. 8 | BULU | (London) | | | |
| B.I. 9 | SALEH | (London) | | | |
| B.I. 10 | KATE | (London) | | | |

SUMATRAN

| | | | | | |
|---|---|---|---|---|---|
| S.I. 1 | SAMPIT | (Yerkes) | S.II. 1 | KANTING | (Yerkes) |
| S.I. 2 | JOWATA | (Yerkes) | S.II. 2 | SANTING | (Yerkes) |
| S.I. 3 | BAGAN | (Yerkes) | S.II. 3 | JINJING | (Yerkes) |
| S.I. 4 | LADA | (Yerkes) | S.II. 4 | TUKAN | (Yerkes) |
| S.I. 5 | DYAK | (Yerkes) | S.II. 5 | SAH | (Yerkes) |
| S.I. 6 | TUPA | (Yerkes) | S.II. 6 | ANAK | (Yerkes) |
| S.I. 7 | BALI | (Yerkes) | S.II. 7 | CHEBEK | (Yerkes) |
| S.I. 8 | TUAN | (Yerkes) | S.II. 8 | AYER | (Yerkes) |
| S.I. 9 | INI | (Yerkes) | S.II. 9 | JIRAN | (Yerkes) |
| S.I. 10 | DATU | (Yerkes) | S.II. 10 | LOKLOK | (Yerkes) |
| S.I. 11 | SUNGEI | (Yerkes) | S.II. 11 | PATPAT | (Yerkes) |
| S.I. 12 | LIPIS | (Yerkes) | S.II. 12 | PETALA | (Yerkes) |
| S.I. 13 | SIBU | (Yerkes) | S.II. 13 | KESA | (Yerkes) |
| S.I. 14 | HENRY | (Bristol) | S.II. 14 | LUNAK | (Yerkes) |
| S.I. 15 | ANN | (Bristol) | S.II. 15 | OSCAR | (Bristol) |
| S.I. 16 | DINDING | (Yerkes) | S.II. 16 | HENRIETTA | (Bristol) |
| | | | S.II. 17 | JULITTA | (Bristol) |

HYBRID MATINGS

| | | | | | |
|---|---|---|---|---|---|
| S.I. 17 | KAMPONG | (Yerkes) | H.I. 1 | KANAK | (Yerkes) |
| B.I. 6 | PADDI | (Yerkes) | H.I. 2 | BEBAS | (Yerkes) |
| B.I. 7 | KYAN | (F.Walton Zoo) | H.I. 3 | SYANG | (London) |
| S.I. 18 | POTTS | (F.Walton Zoo) | | | |
| S.I. 19 | GAMBAR | (London) | | | |
| B.I. 11 | BANTU | (London) | | | |

from crossing Sumatran males with Bornean females, and one hybrid animal (H.I.2) resulted from a cross between a Bornean male and a Sumatran female.

Chromosome analysis.

Of the 27 animals studied, only 8 could be considered to have a normal chromosome constitution following the standard karyotype of this species (Fig. 4.1) given in the Paris Conference (1971); supplement (1975) and following our proposed modification for chromosome 2. Five different chromosome pairs showed variant chromosomes: Nos. 2, 9, 14, 22 and 23. Each of these variant chromosomes will now be described:

Chromosome 2.

As mentioned in the previous section, a subtelocentric variant chromosome 2 was found (Figs. 4.6, 4.9 and 4.12). This chromosome showed a positive G-band at its small short arm, which covered it almost entirely except for the telomere which was faintly stained. The region below the centromere showed two very prominent G-bands and a broad negative band below them. With R-banding, the exact reverse situation was observed: the subcentromeric region and almost the entire short arm were negatively stained, and only the telomere of the short arm occasionally showed a positive R-banded region. The variant chromosome can be derived most simply from the normal chromosome by a single pericentric inversion (Fig. 4.6), one break occurring at the proximal region of band q13 permitting the region pter→q13 to undergo inversion. The rearrangement can be symbolized as inv (2)(pterq13), and the rearranged chromosome as q13/

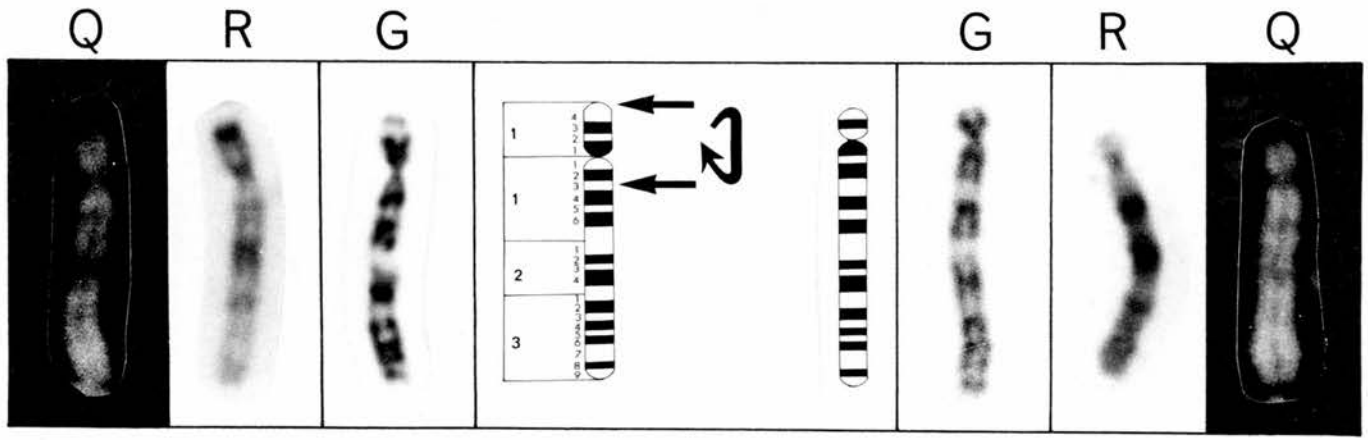


Fig.4.6. Q-, G- and R-banding patterns of chromosome 2 in Pongo pygmaeus. The variant chromosome (right) can be derived from the normal (left) by a pericentric inversion (curved arrow). Straight arrows indicate chromosome break.

q13→pter::q13→qter.

Chromosome 9.

The variant chromosome 9 consisted of a more metacentric chromosome (C.I. = 34.7) which could not be simply derived from the normal chromosome 9. It appeared to have arisen from a complex rearrangement involving three breaks. Break points were identified with both R- and G-banding, and the rearrangement can be viewed as two pericentric inversions, one inside the other, resulting in the rearranged chromosome pter→p13::q21→q13::p13→q13::q21→qter (Fig. 4.7 and 4.8). It would conform to the pattern of rearrangement described as an "inverted insertion" (Paris Conference 1971) and can be described in brief as inv ins(9)(p13q13q21). Q- and C-banding studies confirmed that the normal and the rearranged chromosome 9 differ from all other chromosomes in the karyotype by not showing a C-band at the centromere.

Chromosome 14.

A variant chromosome 14 was found in one animal (S.II.10), (Fig. 4.9). This consisted of a deletion on the entire short arm, thus, del(14)(pter→p11:). C-banding showed a positive heterochromatic region at the centromere. This variant chromosome was never involved in satellite association.

Chromosome 22.

A variant chromosome 22 was found in the same animal (S.II.10), (Fig. 4.9) which also carried a variant chromosome 2. The variant chromosome/

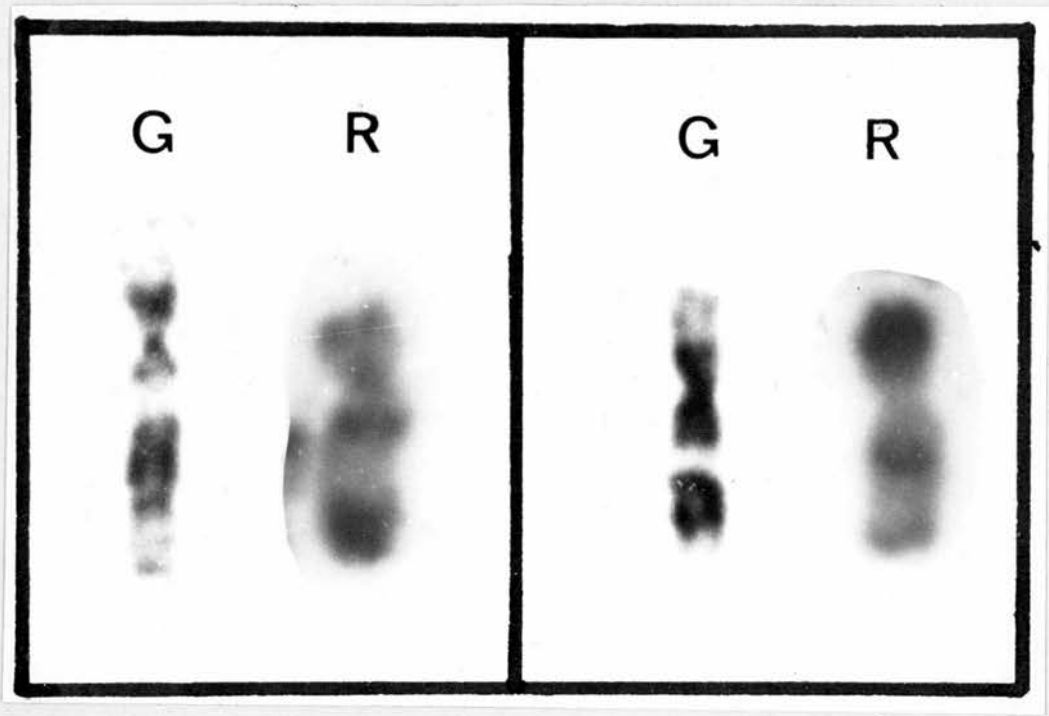


Fig.4.7. G- and R-banding patterns of the normal (left) and variant chromosome 9 (right) in Pongo pygmaeus.

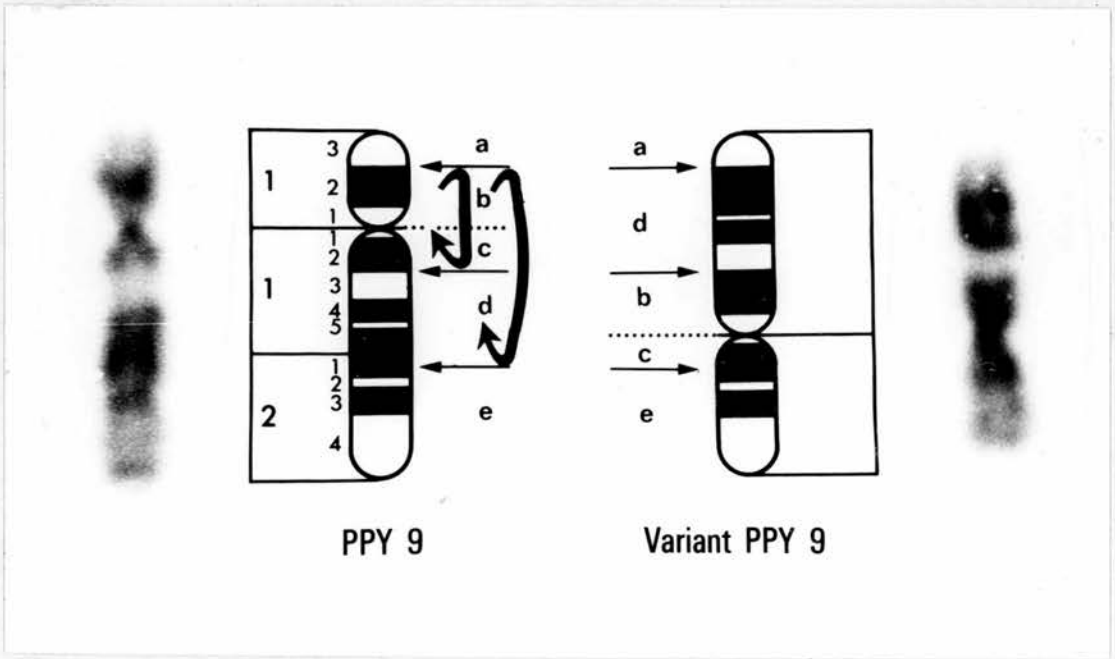


Fig.4.8. Deviation of the variant chromosome 9 from its normal homologue by two pericentric inversions, one inside the other. Straight arrows indicate chromosome breaks; curved arrows symbolise the inversions.

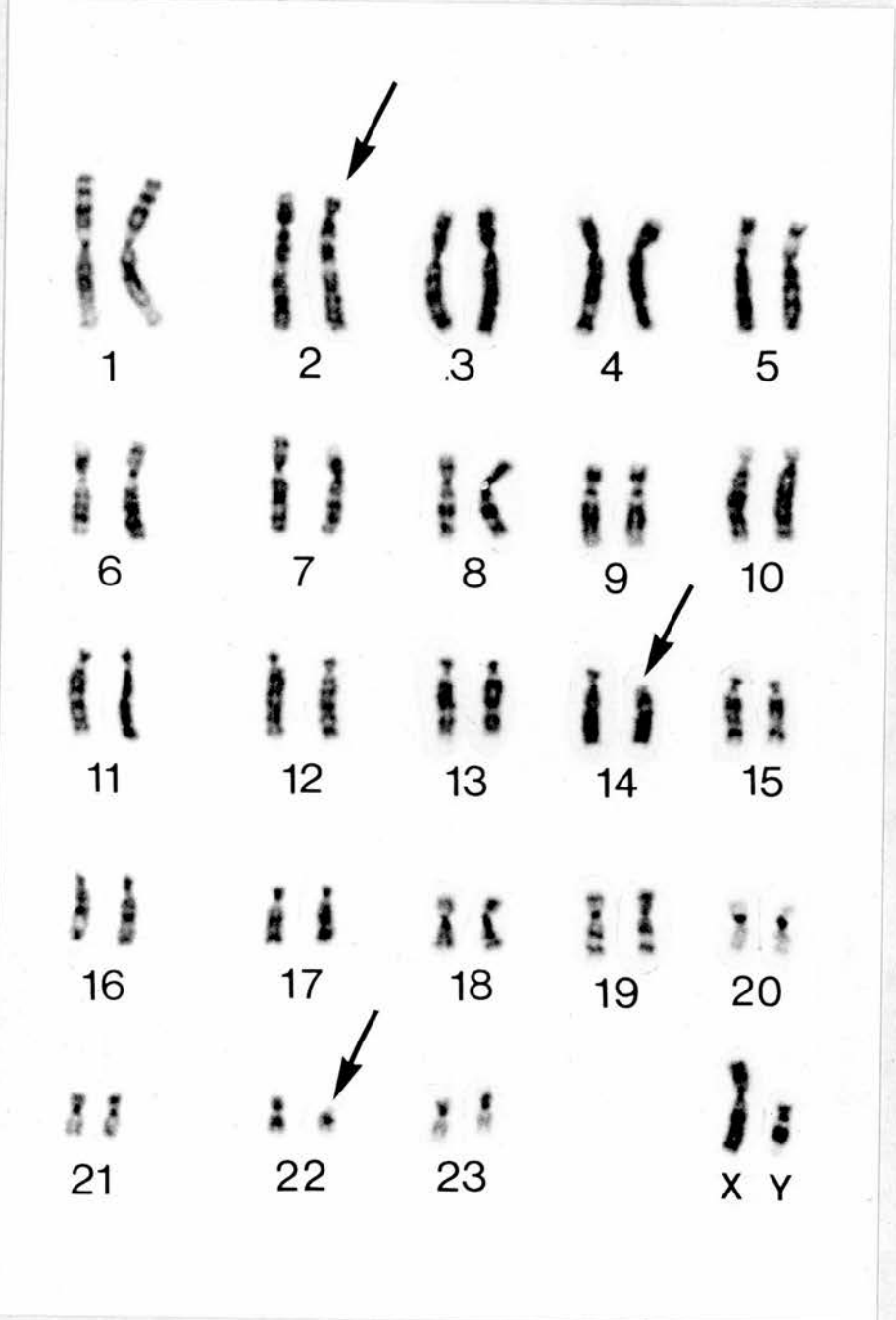


Fig.4.9. G-band karyotype of S.II.10, a carrier of a variant chromosome 2 and deletions in chromosomes 14 and 22.

chromosone 22 showed a deletion of the entire short arm, thus, del (22)pter→p11:). There was no C-band at the centromere, and this chromosome was never involved in satellite association.

Chromosome 23.

A variant chromosome 23 which consisted of a rather metacentric chromosome resulting from the existence of a prominent short arm was found in one animal (S.II.2) (Fig. 4.10). With quinacrine, the region was not brilliant since there is no brilliant fluorescence in this species. With C-banding it was intensely stained (Fig. 4.11). This chromosome was involved in satellite association.

Of the five variant chromosomes described, two of them (Nos. 2 and 9) appeared in a large group of animals, and three of them (Nos. 14, 22 and 23) appeared in isolated individuals (see pedigree of the animals, Fig. 4.5). It is a paradox that the most complex rearrangements, e.g. those involving pericentric inversions, were more widely spread in the animal populations. As illustrated in Fig. 4.5, the rearranged chromosome 2 was found in 11 animals, 8 of which were homozygous carriers and three were heterozygous carriers. This variant chromosome must have been present in the karyotypes of the animals B.I.1, B.I.5, B.I.6, B.I.8, B.I.9, B.I.10 and B.I.11 since they have offspring homozygous for the rearrangement. The variant chromosome might also have been carried by either S.I.8 or S.I.10, B.I.7 or S.I.18, S.I.19 or B.I.12 since it was found in heterozygous condition in their offspring. It is interesting to note that this variant chromosome was found predominantly in Bornean animals/

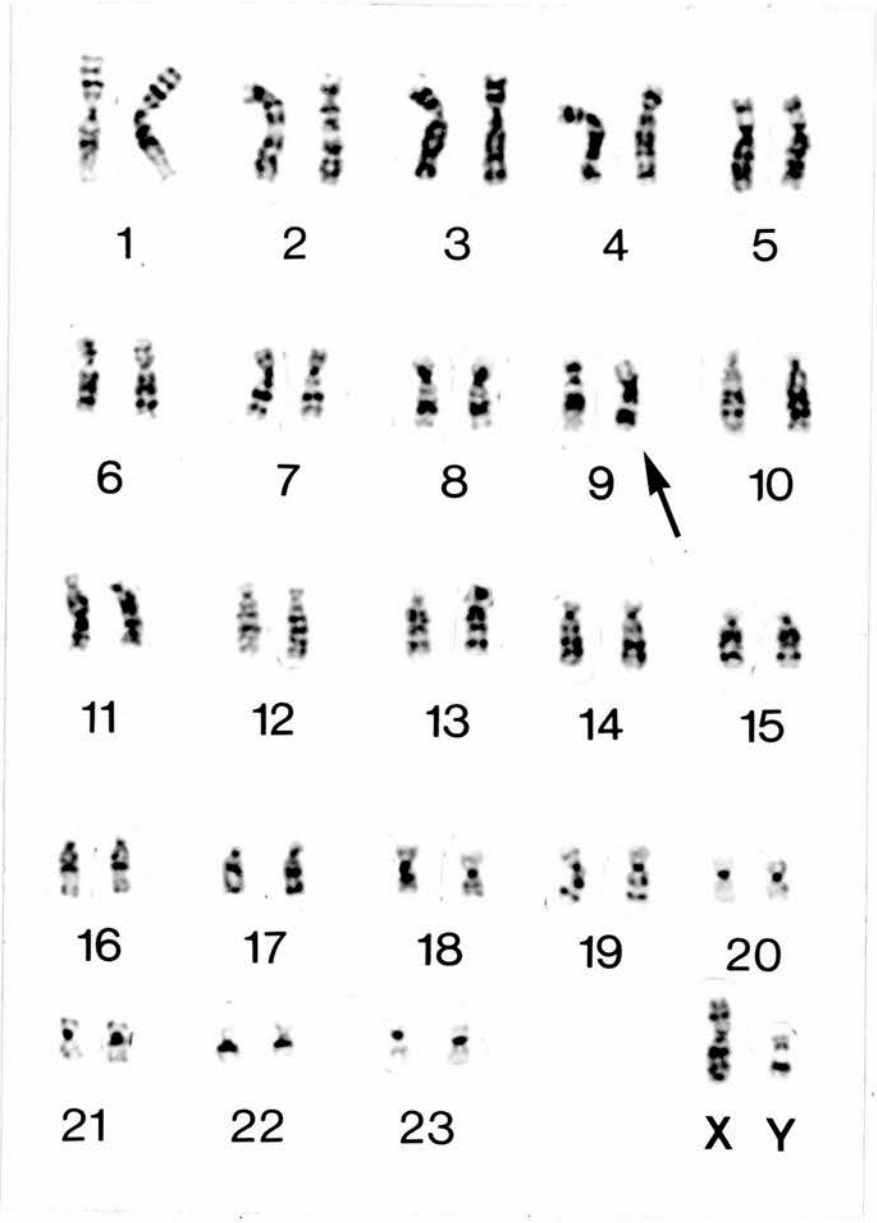


Fig.4.10. G-band karyotype of S.II.2, an animal heterozygous for a variant chromosome 9 (arrowed) and carrier of a variant chromosome 23 (not arrowed; see Fig.4.11).

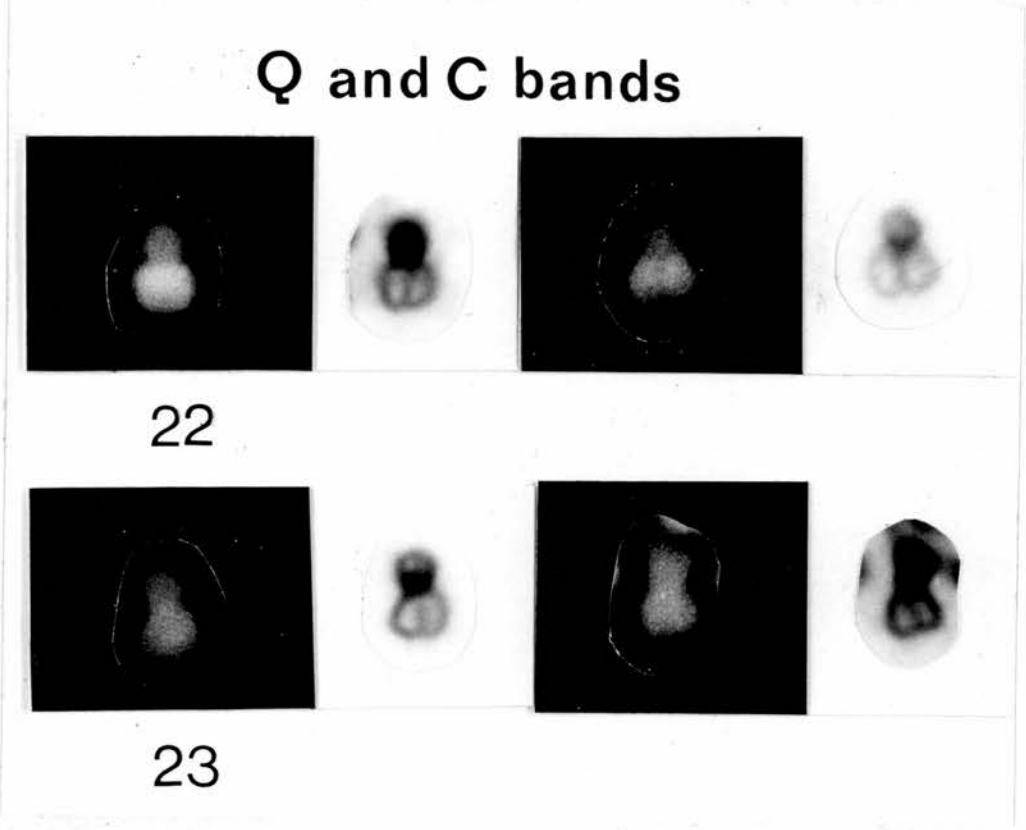


Fig.4.11. Q- and C-banding of chromosome pair 22 and of the normal and variant chromosome 23 in S.II.2.

animals (Figs. 4.12 and 4.13), all those studied being found to be homozygotes. Two hybrid animals show this variant chromosome in heterozygous condition and it is possible that it could have been transmitted by their Bornean parent, although this has not been verified by a chromosome analysis of the parents. The clear exception to this rule is S.II.10 (Fig. 4.9), which is a Sumatran animal, the only one to carry this variant chromosome in the 17 specimens studied from this island.

The rearranged chromosome 9 was found in 12 animals, 9 were heterozygous and 3 were homozygous carriers (Figs. 4.5, 4.10, 4.12 and 4.13). This variant chromosome has also been traced in four other animals: B.I.1, who was a father of an homozygous carrier; S.I.1 who had been mated to a normal female (S.I.2) and was the father of three heterozygous carriers (S.II.1, S.II.2 and S.II.3); S.I.6 who had been mated to a normal male (S.I.5) and was the mother of an heterozygous carrier (S.II.7); and a hybrid (H.I.1) whose father was an homozygous carrier (S. I.17). In the other pedigrees, one or other animal in each of the three pairs B.I.5 and B.I.6, S.I.8 and S.I.9, and S.I.12 and S.I.13 are presumed to be carriers of this variant chromosome, as it has appeared also in their offspring.

(D) Discussion.

Each of the variant chromosomes and their distribution in the populations of orangutan will be discussed separately.

Chromosome 2 and its variant form.

We/

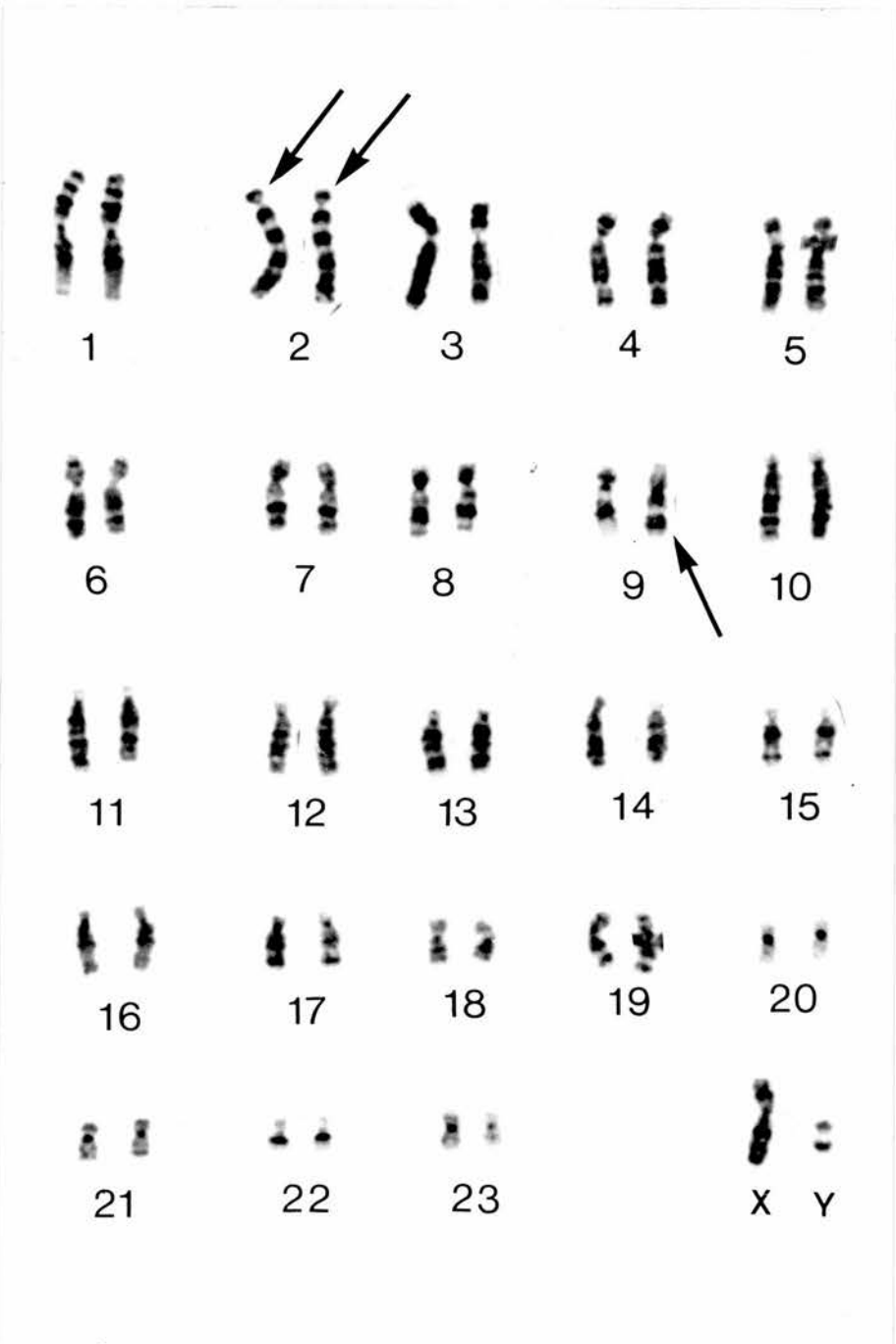


Fig.4.12. G-band karyotype of B.I.3. a homozygous carrier of a variant chromosome 2 and a heterozygote carrier of a variant chromosome 9.

We have defined the "normal" chromosome 2 in Pongo pygmaeus as that which was more frequently found among the animals. It corresponded to a submetacentric chromosome with a centromeric index of 18.9 with two positive G-bands in its short arm and one positive G-band in its subcentromeric region. Sixteen animals were homozygous for this chromosome, and one (S.II.10) had a normal and a variant chromosome 2. The chromosome which we have described as "variant" corresponds to that which is less frequent among the animals. It was carried only in 11animals, 8 homozygote and three heterozygotes. Morphologically, it corresponded to a subtelocentric chromosome with a centromeric index of 10.2, with one positive G-band in the short arm and two positive G-bands in the subcentromeric region.

However, if we consider the two populations separately, all Bornean animals appear homozygous for the "variant chromosome 2", and all the Sumatran animals (except S.II.10) are homozygous for the "normal chromosome 2". Moreover, all the Bornean animals of the adult generation (B.I. 1, 5, 6, 8, 9, 10 and 11) which have not been examined must also be at least carriers of this variant chromosome. Data in the literature in which chromosome banding has been done in orangutans of known origin shows that Bornean animals carry this variant chromosome 2 in homozygous condition (Lucas et al, 1973; Turleau et al, 1975). In the report by Turleau et al (1975) one Bornean animal was mated to a Sumatran animal, and the offspring was heterozygous for the variant chromosome 2 which had been transmitted by the Bornean parent. It is then evident from our own findings and from data in the literature that the frequency of each chromosome/

chromosome type differs between the two populations of orangutan. This raises interesting questions, and the following possibilities must be considered to explain the findings:-

(a) Has this chromosome heteromorphism resulted from a pericentric inversion occurring as a random event in individual animals?

(b) Has the situation arisen by inbreeding and therefore results from studying animals of the same family group?

(c) Do the differences truly correspond to differences in the karyotype of the two subpopulations of orangutan?

(d) Have both chromosome types been maintained as a balanced chromosome polymorphism in the species?

The first possibility (a) is unlikely. Pericentric inversions are rare events, and, although no data is available on their incidence in the orangutan, in man it has been estimated that they occur with a frequency of about 1 in every 10,000 individuals (Hamerton et al, 1975). Pericentric inversion occurring at random would not always affect the same chromosome at the same break points, and there is no logical explanation why they should occur more frequently in one population than in another. Finally, pericentric inversions occurring at random would produce a larger number of heterozygous than homozygous carriers, since the appearance of an homozygous carrier would be an extremely rare event (equal to the square of its probability).

The second possibility (b) is also unlikely, since none of the animals of the adult generation are related. Within each subpopulation the animals were caught on the same island, but at different periods of time, sometimes at intervals of more than 10 years./

years.

The third possibility (c) is the most likely explanation. The maximum likelihood estimates of the chromosome frequencies of each chromosome type have been found from data in Fig. 4.5. In the Bornean group, the estimate for the "variant" chromosome 2 equals 1.00, whereas in the Sumatran group it equals 0.074. This is good evidence that both populations differ significantly in the frequency with which each chromosome type is carried, and strongly suggests that each chromosome type has become fixed in each population. Thus, the chromosome type that we describe as "variant" must be considered the "normal" chromosome 2 in the Bornean subpopulation, and the one we describe as "normal" should only be considered as such in the Sumatran subpopulation. The terms "normal" and "variant" as used previously are only relative to the number of animals studied from each population. Should we have had an excess of Bornean animals, we might well have considered the "variant" chromosome as the normal, and vice versa. For this reason, we would prefer to use the term "Bornean chromosome 2" and "Sumatran chromosome 2" rather than "variant" and "normal".

The fourth possibility (d) is unlikely. A balanced chromosome polymorphism would need to confer some selective advantage to the heterozygous over both homozygous types, and this is not the case in these animals. In fact, heterozygous animals are probably very rare since only one in 25 animals was a heterozygous carrier (S.II.10) (Fig. 4.9),and all other animals were homozygous for one or other of the chromosome types within each subpopulation.

Assuming that our observations reflect differences in the karyotype between Bornean and Sumatran specimens of orangutan, two important/

important questions arise. The first is about the origin of the Bornean and Sumatran chromosome 2 in relation to the phylogeny of the chromosomes of Pongo pygmaeus. The second is about the possible mechanisms by which the new chromosome type spread and became fixed in one population. To answer these questions, it is necessary to understand the geographical distribution of Pongo pygmaeus. In the past, this species was widely spread in South-east Asia and the fact that at the present time this species can be found in two islands (Borneo and Sumatra) is a consequence of the fact that both regions were once connected by land. The South China sea which lies between Borneo and Sumatra is a shallow sea, and the level of water fluctuated considerably between glacial and interglacial periods. When the sea basin was dry, it was possible to pass from one region to the other and this is why the fauna of Sumatra resembles that of Borneo more closely than that of Java, although Java is geographically closer to Sumatra. It is believed that at the end of the last glacial period, approximately 8,000 years ago, the basin of the South China sea was filled with water, and from then until now, Borneo and Sumatra have been isolated from each other. Thus, the original population of orangutan was split into two subpopulations which became geographically isolated. Considering 9½ years the average generation time of this species, both subpopulations have thus been in geographical isolation for a period of approximately 800 generations.

It is not unreasonable to think that one of these two chromosome types may be the ancestral chromosome from which the other chromosome was derived. Since each chromosome may be derived from the other by a simple pericentric inversion it is impossible to know precisely which/

which chromosome was the original and which one was derived. However, a comparison with the karyotype of man, the chimpanzee and the gorilla might help to elucidate this problem. Chromosome 2 in the orangutan is homologous to chromosome 3 in man (HSA 3), to chromosome 2 in the chimpanzee (PTR 2), and to chromosome 2 in the gorilla (GGO 2). Of the four species, it is only the orangutan that differs in this chromosome pair. HSA 3, PTR 2 and GGO 2 are all metacentric chromosomes and show practically the same morphology and G-banded patterns. If we assume that the Homininae and the Ponginae are phylogenetically related taxa, as it is generally accepted, the common ancestor of both most probably has one chromosome from which HSA 3, PTR 2, GGO 2 and PPY 2 were all derived. Which of these two different forms of PPY 2 is more likely to be the original chromosome 2 in Pongo pygmaeus? The most likely is the one that can be most simply derived from HSA 3, PTR 2 and GGO 2 according to the principle of parsimony. This principle assumes that a network of descent can best be explained with the fewest number of changes. In the case of PPY 2, we can reconstruct chromosome 3 in man and its homologue in the chimpanzee and the gorilla from either one of the chromosomes described. However, only two chromosome breaks and one pericentric inversion are needed if derivation is from the Bornean chromosome 2 (Fig. 4.14) while more than two breaks are needed to reconstruct HSA 3, PTR 2 and GGO 2 from the Sumatran chromosome 2. This suggests that the original chromosome 2 of Pongo pygmaeus is probably the one that is carried Bornean animals, and that the one carried by Sumatran animals has been derived from it by a pericentric inversion.

The second question about the mechanism by which the derived chromosome/

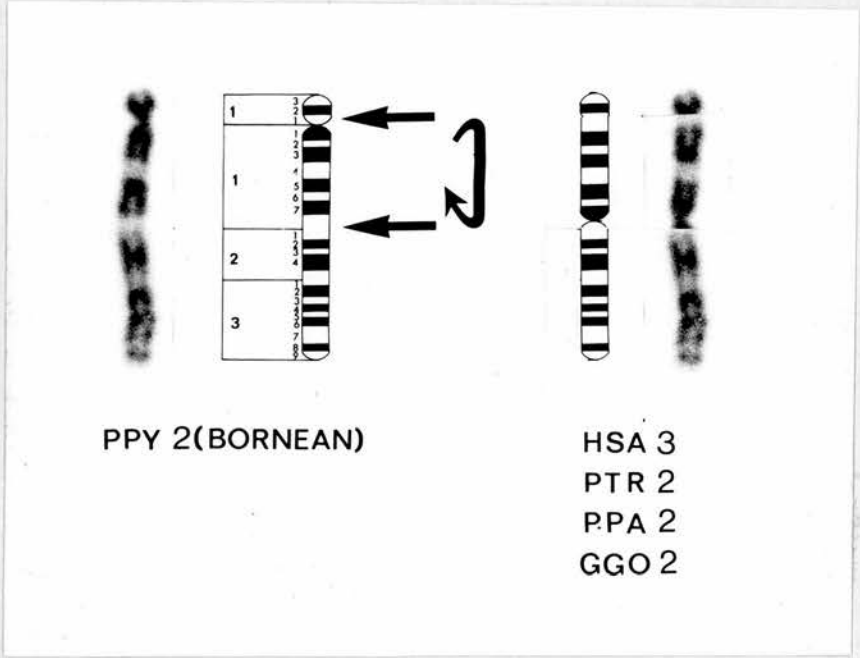


Fig.4.14. Deviation of chromosome No.3 in man and No.2 in Pan and Gorilla by a single pericentric inversion from the Bornean chromosome 2 in Pongo pygmaeus.

chromosome became fixed in Sumatra and the original chromosome became fixed in Borneo is intriguing. In the first place we do not know when the derived chromosome appeared in this species, whether before the splitting of both populations or whether after geographical isolation. If the maximum likelihood estimates of the frequencies for each chromosome were truly accurate, this would mean that all Bornean animals are homozygous for the original chromosome 2 (or the Bornean type), and that the great majority (but not all) of the Sumatran animals would be homozygous for the derived chromosome 2. Our numbers are small, and a larger study of animals would be needed to solve this problem, but if these findings were confirmed, the derived chromosome 2 has probably appeared in the Sumatran population after it became geographically isolated from the Bornean population. Anyway, whether emerging before or after geographical isolation, it is clearly evident that genetic drift and/or strong selection has been operating to establish such extreme differences in the frequency of both chromosome types in each population. Random drift could have occurred if the original population where the new chromosome was formed was small. There are no data on the numbers of orangutans that inhabited the islands of Sumatra and Borneo in the past; all that we know is that present populations are around 4,000 animals in Borneo and only 100 in Sumatra (Napier and Napier, 1967). Although there is no doubt that both populations were considerably larger in the past, it is still possible that they could have been small enough for genetic drift to operate within them. A high degree of inbreeding resulting from the social organization of the species could also have/

have played an important role in enhancing genetic drift in a small population. On the other hand, selection must also be considered, assuming that it has probably operated in opposite directions in both populations. If, after an initial period of spreading inside the population, the derived chromosome became so frequent as to allow for two heterozygous carriers to mate, homozygous carriers for both chromosome types would be produced. If, during this process, selection favoured one homozygous type (e.g. the homozygote for the derived chromosome) against the other homozygous type and the heterozygous carrier, the derived chromosome would gradually become fixed in the population, and the number of generations needed for this to happen would depend on the intensity of selective pressure. Thus, heterozygotes would become more rare, and one of the homozygous types would be eliminated in the population. This situation seems to be operating in Sumatran animals in which the incidence of heterozygous carriers appears to be very low. (The other two heterozygous carriers found by us are hybrid animals, and they do not represent the normal situation found under natural conditions where Bornean and Sumatran animals can never mate).

Selection against heterozygous carriers is easy to understand since heterozygous carriers for a pericentric inversion may produce unbalanced gametes as a consequence of crossing over within the inversion loop at meiosis. Fertility may therefore be lower in heterozygotes, and this is a rule in most organisms, although exceptions may exist (see next section of this discussion on chromosome 9 in this species). The best evidence for selection against heterozygosity would be to assess the fertility of heterozygous individuals/

individuals and to compare it with that of the homozygous animals. Unfortunately this cannot be done with the small number of animals available for study, and in any case, all heterozygous animals of this group are prepubertal. Selection against one homozygous type could result if environmental conditions changed in such a way that only the homozygous carriers for the alternative type could survive. According to White (1973) this could have occurred as a consequence of cross over reductions in the heterozygous carriers for a pericentric inversion. Any mutation occurring at the regions involved in the chromosome rearrangement would be carried by the chromosome in which it occurred, and would not be recovered as a viable product if it passed to its homologous partner by crossing over. Thus, mutations will be accumulated by each chromosome type and this would make them both morphologically and genetically different. When homozygous animals are formed, these homozygous types would differ both genetically and in their chromosome constitution. Selection may favour the survival of one of these homozygous types, and eliminate the other. This might well be the mechanism by which the two subpopulations of orangutan show such extreme differences in the frequency for their chromosome 2, and might well be the principal mechanism to explain the phylogeny of the chromosomes of the great apes and man from a common ancestor.

Chromosome 9 and its variant form.

The chromosome complement of Pongo pygmaeus has one chromosome pair with identical morphology and G- (or R-) banding pattern to chromosome No.12 in man, and this chromosome has been designated as No.9/

No.9 in the karyotype of Pongo pygmaeus (Paris Conference (1971); supplement (1975) . Variants of chromosome 9 have been described in the literature (although they have often been designated with different criteria of nomenclature) by Lucas et al (1973), Dutrillaux et al (1975b), Turleau et al (1975) and Seuanez et al (1976a and b). A detailed study of these reported variant chromosomes indicates that they all represent an identical rearrangement involving chromosome 9. This must therefore be a common type of variant chromosome with a high incidence in the population of Pongo pygmaeus. Our own findings show that the variant chromosome 9 was found in 12 animals, four of Bornean and 8 of Sumatran origin. The variant chromosome was also traced in four other animals not studied by us. Of the 12 carriers of the variant chromosome, 3 were homozygous, and 9 were heterozygous. The three possible combinations (homozygous for a variant 9, heterozygous for a variant 9, and normal homozygous) could be found in both Bornean and Sumatran populations.

The fact that this variant chromosome has been found in the two subpopulations of orangutan with a high incidence raises the same kind of questions as were raised in the previous section when discussing both types of chromosome 2 in this species. The possibility that this variant chromosome could have appeared as a random event in so many individuals is obviously unlikely, since the rearrangement is too complex and its incidence too high. The second possibility is unlikely as well, since the animals of the adult generation are apparently not related, and, moreover, those coming from Borneo have no chance of being related to those from Sumatra.

The/

The third possibility is also unlikely, since this variant chromosome is found in both populations of orangutan, and the three possible combinations are found in each group. The fourth possibility is the most likely explanation. A genetic trait which occurs in a breeding population with a higher incidence than would be maintained by recurrent mutation may be defined as a polymorphism (Ford, 1940). The complex rearrangement giving rise to this variant chromosome 9 must have occurred before the original population of orangutan spread into both Borneo and Sumatra, a period of at least 8,000 years, when both populations became effectively isolated. The fact that this rearrangement has an ancient origin and the variant chromosome has a widespread distribution suggests that it has been maintained in both subpopulations as a balanced polymorphism. For this to happen, we would expect that some advantage must exist for heterozygous carriers, although this is difficult to prove. We should note, however, that this rearrangement would,in the heterozygous state, result in the formation of two inversion loops at the first meiotic division, as illustrated in Fig. 4.15. Single, and most double, cross overs in such a bivalent would result in the formation of unbalanced products of the duplication - deficiency or dicentric type. However, if crossing over were confined to the terminal segments of the chromosomes (segment "a" and segment "e" in Fig. 4.15) this would not affect the production of balanced gametes, and the situation would be compatible with normal fertility. We know that in the C group chromosomes of man, among which we find the homologous human chromosome (No.12) to PPY 9, most chiasmata are indeed found in the terminal segments of the chromosome/

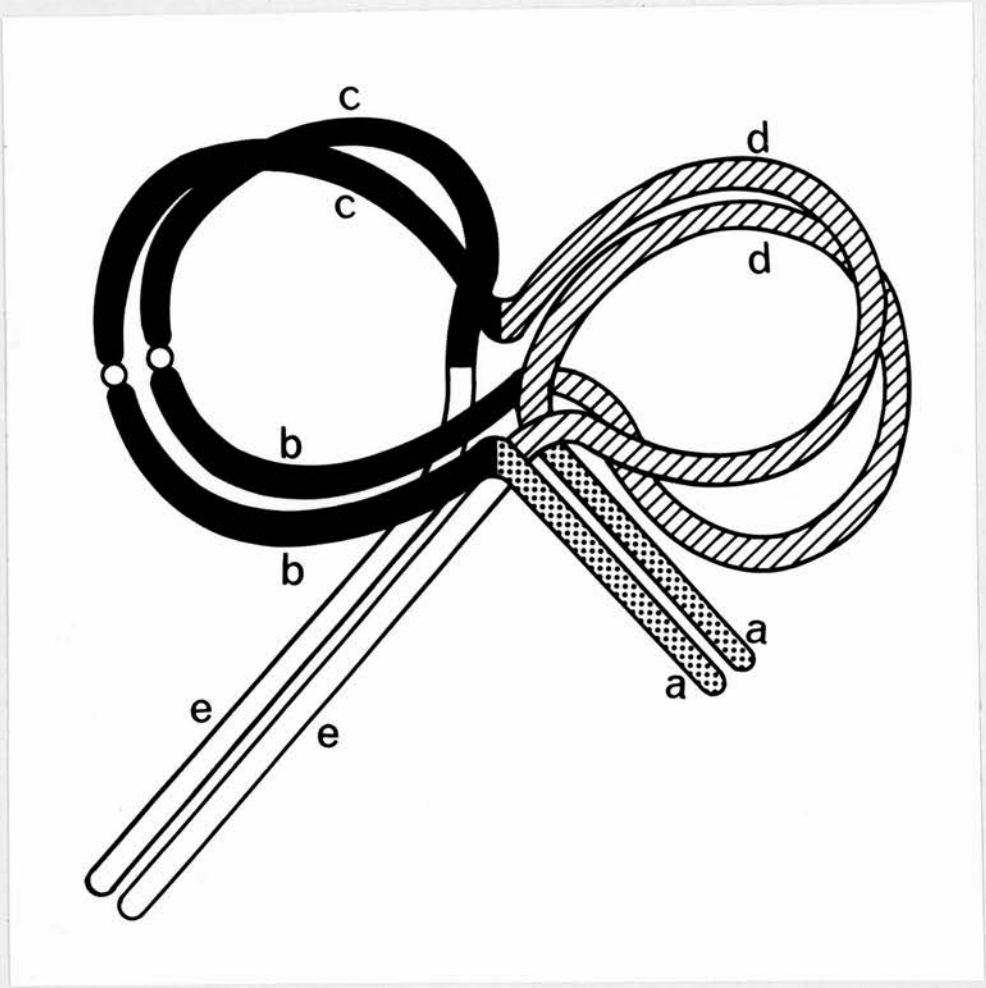


Fig.4.15. Theoretical configuration of bivalent 9 in a heterozygous carrier. Regions a to e are the regions illustrated in Fig.4.8. Cross-overs at regions a and e will result in balanced products. A single cross-over at region b or c would result in unbalanced products of the duplication-deficienty type. A single cross-over at region d would result in unbalanced products, carrying dicentric chromosomes and acentric fragments.

chromosome arms (Hulten, 1974). If this were the case in Pongo, and if chiasma position is an accurate reflection of cross-over site, then this preferential location of chiasmata might well contribute to the maintained fertility of the heterozygous carriers. There is at least one report in the literature (Turleau et al, 1975) in which an heterozygous animal for a variant chromosome 9 was mated to a normal homozygous carrier for chromosome 9, and the variant chromosome was transmitted to two offspring.

In mammals in general, Robertsonian translocations are frequently found as chromosome polymorphisms (White, 1973), but other types of polymorphic chromosome rearrangements are less frequent. There is evidence, however, that pericentric inversions may be polymorphic in populations of the deer mouse (Ohno et al, 1966; Arakaki et al, 1970); plains wood rat (Baker et al, 1970); South American populations of Rattus rattus (Bianchi and Paulette-Vanrel, 1969); mountain voles (Gileva and Pokrovski, 1970); African pygmy mice (Matthey and Jotterand, 1970, Jotterand, 1972); and the European shrew (Ford and Hamerton, 1970).

In the Hominidae, it has been suggested (Bobrow and Madan, 1973; de Grouchy et al, 1973) that pericentric inversions are the major kinds of rearrangements within the group, and clear examples of homologies between chromosomes differing by an inverted pericentric region are evident (Paris Conference (1971); supplement (1975) . Such a mechanism has been found to explain the difference between the chromosome 2 in Bornean and Sumatran animals, a situation which has led to established differences in the karyotypes of both subspecies of orangutan. In the case of chromosome 9, however, pericentric inversions have been responsible for producing a variant chromosome/

chromosome type which is widely distributed in both subpopulations and has no effect in producing a variety or a subspecies within Pongo pygmaeus.

In man, obvious pericentric inversions involving whole or partial C-band regions occur with relatively high frequencies in at least two chromosomes in the complement (Buckton et al, 1976). These human inversions, however, are small, and appear to be confined to regions occupied by constitutive heterochromatin - such regions being considered of low genetic activity - and are not therefore comparable to the situation described here in Pongo pygmaeus. On the other hand, there are reports of larger pericentric inversions in man which involve euchromatic chromosome regions, but these are often associated with pathological conditions. In some less common cases, these larger pericentric inversions have been found to be transmitted by phenotypically normal heterozygotes who showed normal fertility (Jacobs et al, 1967; Weitkamp et al, 1969; Crandal and Sparkes, 1970; Betz et al, 1974; de la Chapelle et al, 1974; Jacobs et al, 1974), but the incidence of chromosomes with such inversions in human populations is extremely low. Moreover, in all cases ascertainment was through some kind of clinically abnormal condition, and in only one (Betz et al, 1974) was the inversion present in the homozygous state. Thus in man, the best studied of all hominoids, none of the pericentric inversions that involve significant amounts of euchromatic material can be considered to be a fixed balanced polymorphism in the population. Whether rearrangements of the type revealed in our own study of the orangutan are to be found in other hominoid species, however, remains an open question.

Variant/

Variant chromosome 14.

Chromosome 14 of Pongo pygmaeus is homologous to chromosome 13 in man, showing an identical morphology and banding pattern. The only difference between these two species is that the region of the short arm satellite in man may show brilliant fluorescence, while in Pongo it never does. In the orangutan, the short arm satellite region of chromosome 14 is: (a) involved in satellite association, (b) positively stained by the Ag-AS technique (Tantravahi et al, 1976), (c) a site of hybridisation with cRNA to the 18S and 28S rDNA cistrons (Henderson et al, 1976a), and (d) a site of positive hybridisation with cRNA to human DNA satellite I, II and III (Gosden et al, 1977; Mitchell et al, 1977). Chromosome 13 in man is similar in all four respects except that its short arm region is a site of hybridisation with cRNA I, II, III and IV (Gosden et al, 1975).

Deletions of the short arm of chromosome 14 in the orangutan have not been reported, although this region is considered variable in length as is the short arm of chromosome 13 in man [Paris Conference (1971); supplement (1975)]. Such variations in the length of the short arm of chromosome 13 in man occur in normal individuals but have no apparent phenotypic effect. Moreover, chromosome 13 in man is frequently involved in centric fusion rearrangements notably with chromosome 14. Robertsonian translocations are the most common translocations in man occurring with an incidence of 0.09% (Hamerton et al, 1975). A great number of such translocation carriers have been found to be phenotypically normal, and the translocation has been discovered through an unbalanced/

unbalanced proband. In some less common cases, deletion of the short arm of chromosome 13 in man is accompanied by a clinical condition (de Grouchy et al, 1966; Emerit et al, 1968). It has been suggested that such situations arise because the homologous region in the normal chromosome carries deleterious recessive genes which are allowed to express themselves in hemizygous state as a consequence of the deletion. This condition in man is, however, rare, and our findings in the orangutan resemble the more common situation in man where no known phenotypic effect is produced.

Variant chromosome 22.

The normal chromosome 22 in Pongo pygmaeus is identical in morphology and banding patterns to chromosome 21 in man, except that in this latter the short arm satellite region may fluoresce brilliantly. Both in the orangutan and in man the short arm of these chromosomes has the properties (a), (b), (c), and (d) mentioned for the short arm of chromosome 14 in the orangutan (see previous section).

In the two species, the short arm of PPY 22 and HSA 21 is variable in size [Paris Conference (1971); supplement (1975)]. In man, short arm deletions of chromosome 21 (and also 22) have been found to occur in ring G chromosomes(Nance and Engel, 1967; Cantu et al, 1975), and these situations usually produce clinical abnormalities in their carriers. However, loss of the short arm of chromosome 21 usually occurs when D/G translocations are formed in man, and most balanced translocation carriers are phenotypically normal.

In/

In the orangutan, we have found one animal (S.II.10) which is a carrier of two deletions and one pericentric inversion. The only visible abnormality was that both deleted chromosomes did not participate in satellite association. In Pongo pygmaeus all acrocentric chromosomes (9 chromosome pairs) are involved in satellite association and their short arm satellite regions contain 18S and 28S rDNA cistrons (Henderson et al , 1976a). These two deletions must have eliminated part of these highly repeated DNA sequences from the genome of this animal, and the fact that multiple copies are still carried by other chromosomes probably makes this loss of no significance. A comparable, although not identical situation is found in man in deletions of this kind, since in man there are 5 pairs of acrocentric chromosomes in which these DNA sequences are carried. In the gorilla, however, a deletion of the entire short arm of chromosome 14 is of no significance, as we have already reported in the previous chapter, but a deletion of the entire short arm of chromosome 22 might have a totally different effect, since in this species the 18S and 28S cistrons are located in only two chromosome pairs (GGO 22 and 23), and deletions of this kind may be intolerable.

Variant chromosome 23.

A variant chromosome 23 was found in one animal (S.II.3) and this consisted of an increase in length of the short arm which transformed the chromosome into a metacentric. This region is positively stained with C-banding, and it was found to contain highly repeated DNA sequences homologous to human satellite DNA I, II and III in a larger amount than its normal homologue (Gosden et al, 1977/

1977; Mitchell *et al*, 1977). This variant chromosome was also involved in satellite association. This suggests that the increase in length of the short arm has probably been accompanied by an increase in the amount of highly repeated DNA sequences with no appreciable phenotypic effect in the carrier. An increase in the length of the short arm of chromosome 23 has probably occurred in the line leading to *Pan paniscus* after it split from the common stock with *Pan troglodytes*. In these two species, however, as in chromosome 22 of man and 23 of the gorilla, the short arm region of this chromosome pair (No. 23) may show brilliant fluorescence.

(E) Summary Of This Chapter.

The study of the chromosome complement of the orangutan has shown the following:-

(1) Chromosome morphology and banding patterns of pair No.2 was found to be different between the Sumatran and Bornean subpopulations of orangutan. Each subpopulation has its own chromosome type which has become fixed with a high frequency, probably as a result of genetic drift and/or selection. The terms "normal" and "variant" when applied to these chromosome types should be dropped, and the terms "Sumatran" and "Bornean" are preferable to identify these two chromosome types. The Bornean chromosome 2 is probably the original chromosome 2 of the species and the Sumatran chromosome 2 might have arisen by a pericentric inversion.

(2) A complex chromosome rearrangement consisting of two pericentric inversions, one inside the other, was found to be maintained as a balanced chromosome polymorphism in the two subpopulations of orangutan. The rearrangement affected chromosome 9, the only chromosome pair in the orangutan with no C-band region at its centromere. Such a balanced polymorphism must be at least 8,000 years old, a period encompassing roughly 800 generations. This is good evidence that pericentric inversions may spread successfully in the population and not necessarily be restricted by infertility barriers.

(3) Short arm deletions affecting chromosomes 14 and 22 were found in one animal which in addition had an heteromorphic pair No.2

(a/

(a Sumatran and a Bornean chromosome No.2). None of these variant chromosomes produced appreciable phenotypic effects.

(4) Length increase in the short arm of chromosome 23 occurred in one animal probably as a result of an increase of constitutive heterochromatin. No appreciable effects were found in the carrier.

(5) The morphology and the G- Q- and C-banding pattern of the Y chromosome of this species is described.

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# CHAPTER 5: LATE REPLICATION PATTERNS IN THE CHROMOSOMES OF MAN AND THE GREAT APES.

## (A) Introduction.

The development of techniques capable of demonstrating the sequence of DNA replication in individual chromosome bands (Latt, 1973; Perry and Wolf, 1974) has permitted the identification of regions of DNA replication at the chromosomal level with a greater precision than was possible using autoradiographic techniques. A comparative study of the pattern of late DNA replication of the chromosome complement of man and three species of great ape: Pan troglodytes, Gorilla gorilla and Pongo pygmaeus using these tehcniques permits us to enquire:-

(i) Whether the regions of late DNA replication in the chromosome complement of the great apes coincide, as in man, with regions of positive Q- G- and C-band regions (Ganner and Evans, 1971; Calderon and Schnedl, 1973; Latt, 1973, 1975; Epplen et al, 1975; Grzeschick et al , 1975), and

(ii) whether there are similarities in the pattern of late DNA replication between chromosomes of different species showing similar Q- G-banding patterns.

Detailed information on the techniques used is given in Section III (Appendix), but basically, cells were cultured following two different protocols:-

(i) in a medium containing BUdr for 48 hours, except for the last five hours when cultures were pulsed with thymidine (T-pulsed cultures), and

(ii) in a medium containing thymidine for 72 hours, except for the last/

last five hours when cultures were pulsed with BUdr (B-pulsed cultures).

(B) Results.

T- and B-pulsed cultures were obtained in man, Gorilla gorilla and Pongo pygmaeus. However, in Pan troglodytes preparations were unobtainable for T-pulsed cultures because of technical difficulties. Chromosome preparations from T-pulsed cultures showed a pattern in which intensely stained regions coincided with Q- G- and C-band regions, whereas the same regions appeared palely stained in chromosome preparations from B-pulsed cultures (Fig.5.1). The quality of cytological preparations was generally better in T-pulsed cultures, except that the number of cells was lower than in B-pulsed cultures as a consequence of the shorter incubation period, and presumably by the action of the thymidine analogue (BUdr) for the first 43 hours. However, T-pulsed cultures produced chromosome preparations which were more sharply stained with Giemsa with a better definition between intense and pale Giemsa bands than B-pulsed cultures. This effect was particularly noticeable in two kinds of region: (i) the short arm-satellite region of the acrocentric chromosomes, which appeared elongated and despiralized in B-pulsed cultures, and (ii) the terminal regions of many chromosomes of Gorilla gorilla. In T-pulsed cultures the distal tips of the chromosome arms appeared sharply defined, whereas in B-pulsed cultures these regions were paler than the rest of the chromosome (Fig.5.1). The technique used to stain chromosome preparations with Giemsa (Perry and Wolf, 1974) gives better definition than that used by Latt (1973, 1975) with Hoechst 33258 fluorescence, and a greater number of late replicating regions can be observed in the chromosomes (Fig.5.2, 5.3, 5.4, and 5.5). In the human chromosome complement, we/

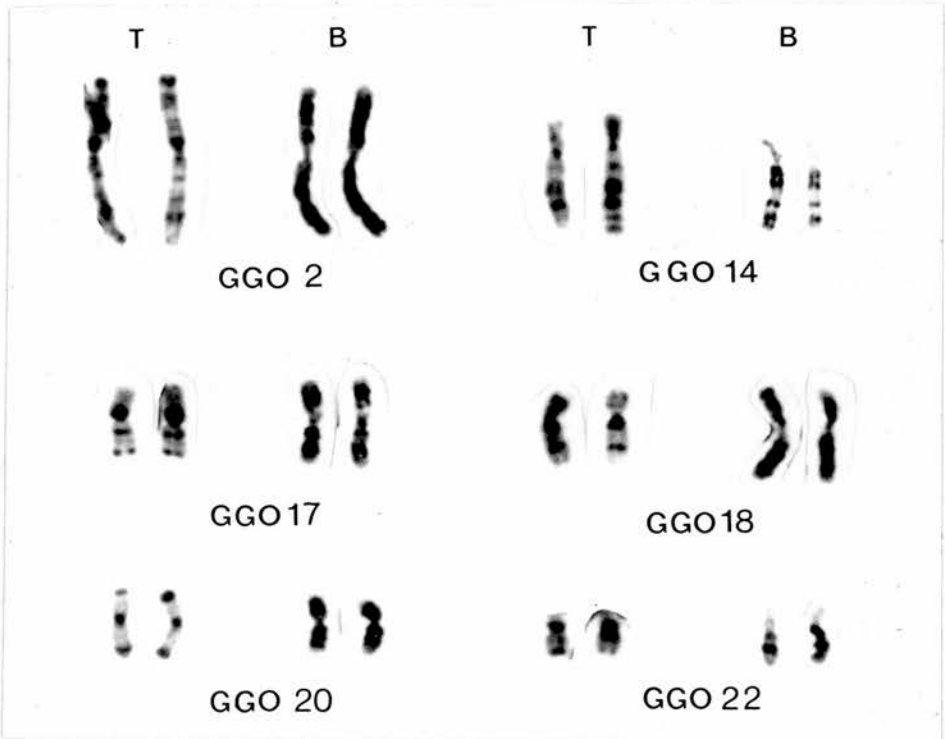


Fig.5.1. Comparison between patterns of late DNA replication between T- and B-pulsed cultures. Regions of intense Giemsa staining in T-pulsed cultures correspond to pale regions in B-pulsed cultures.

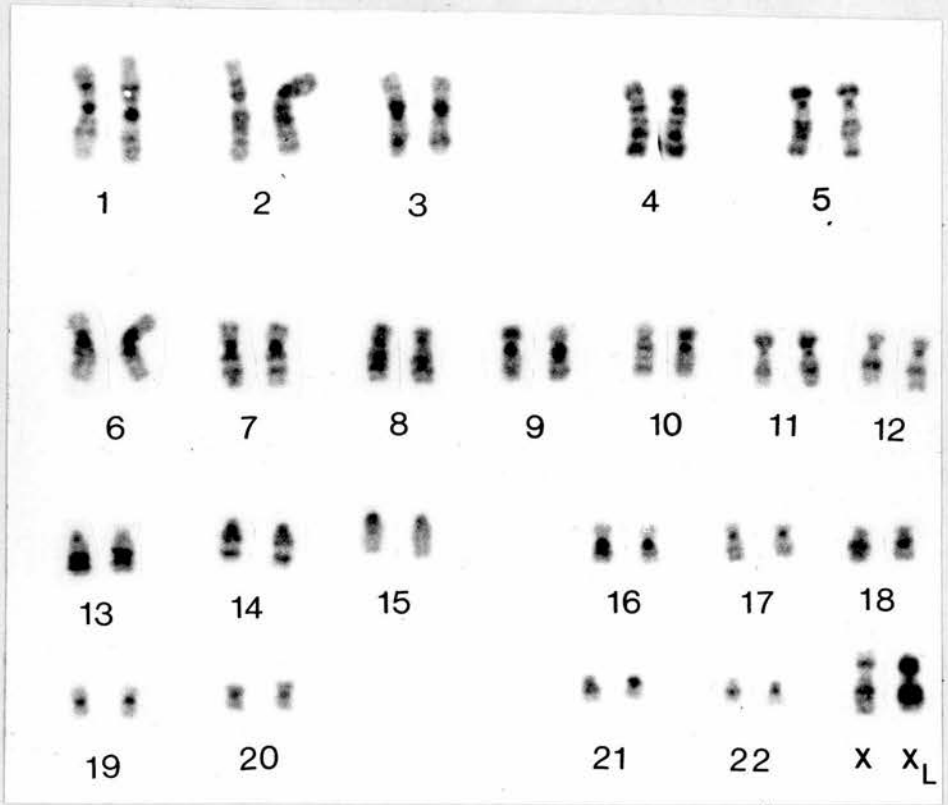


Fig.5.2. Late DNA replicating regions of the human chromosome complement (T-pulsed cultures) showing coincidence with G- and C-band regions. Note the difference between the normally replicating X chromosome (X) and the late replicating X chromosome ($X_L$).

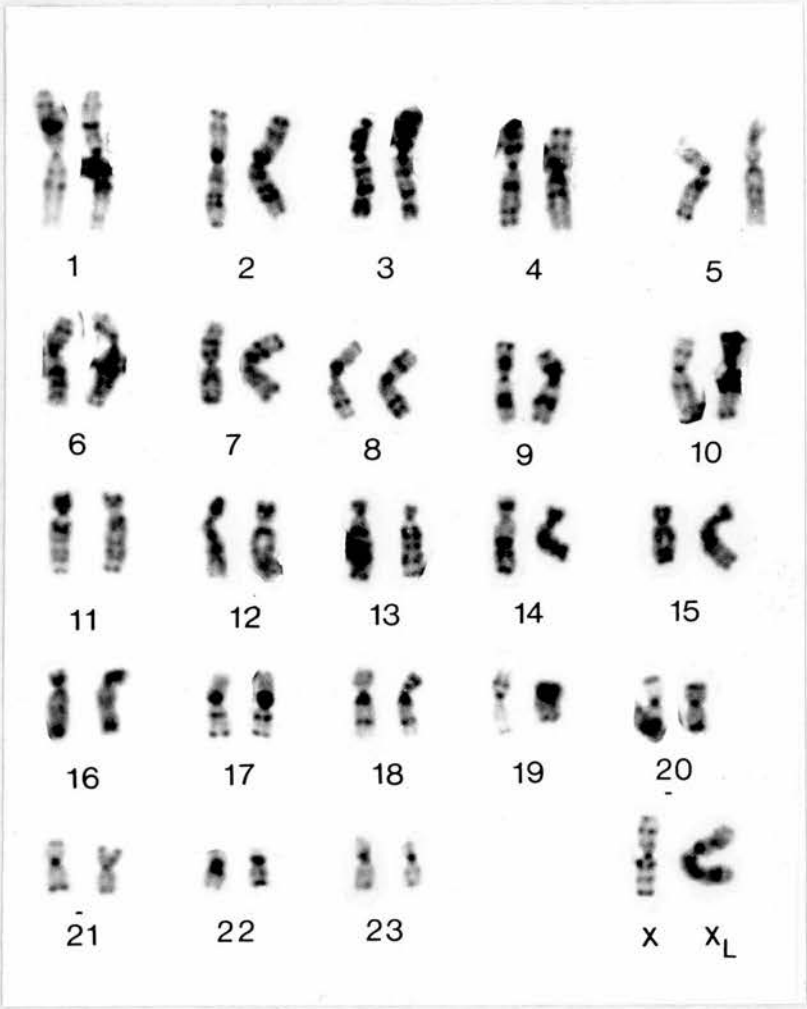


Fig.5.3. Late DNA replicating regions of the chromosome complement of Gorilla gorilla (T-pulsed cultures) showing coincidence with G- and C-band regions. Note the difference between the two X chromosomes.

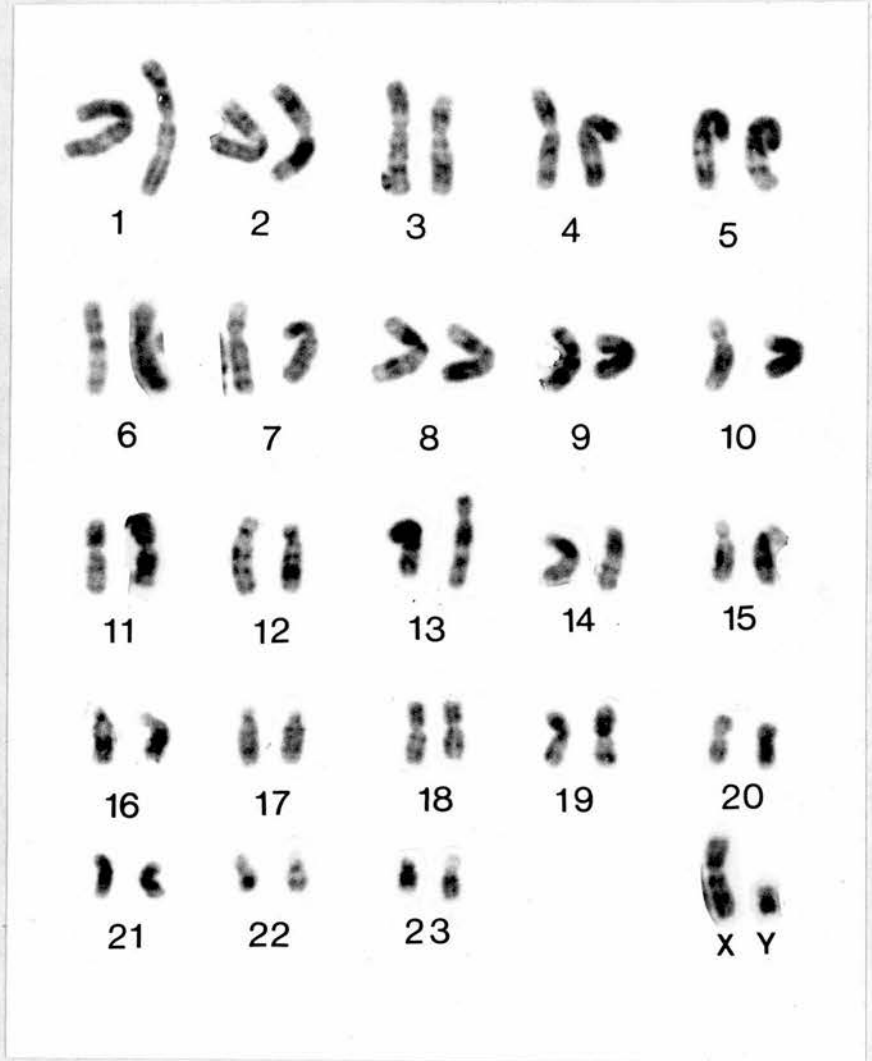


Fig.5.4. Late DNA replicating regions of the chromosome complement of Pan troglodytes (B-pulsed cultures) showing coincidence with R-band regions.

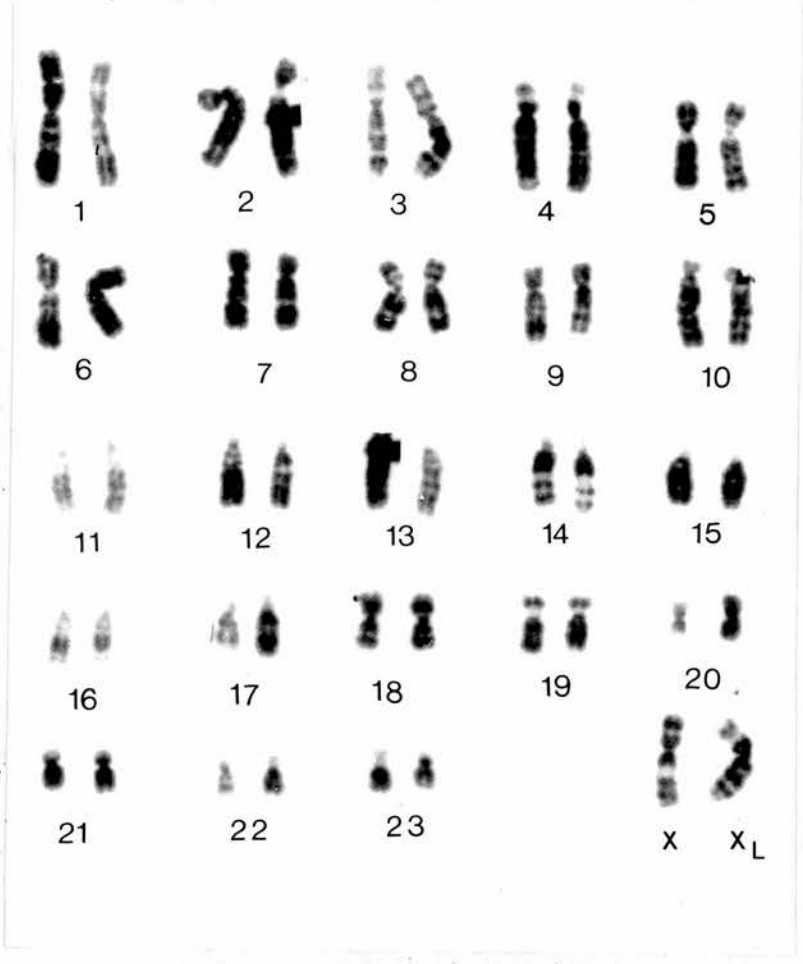


Fig.5.5. Late DNA replicating regions of the chromosome complement of Pongo pygmaeus (B-pulsed cultures) showing coincidence with R-band regions. Note the difference between the two X chromosomes.

we found that the number of late DNA replicating regions and their location basically corresponded to those previously reported by Grzeschik et al (1975) in cell cultures studied under identical experimental conditions. The regions stained with Giemsa corresponded to positive Q- G- and C-band regions of the human chromosome complement, although not all Q- and G-band regions were late replicating. A comparison between the late replicating pattern of the human chromosome complement and that of the great ape species is shown in Fig. 5.6 a-h. The diagram shows chromosome homologies between man and Pan troglodytes, Gorilla gorilla and Pongo Pygmaeus as described in the Paris Conference (1971); supplement (1975). Some minor modifications have been introduced to this diagram. Chromosome 2 in Pongo pygmaeus corresponds to a Sumatran chromosome 2 (see Chapter 4), chromosome 18 of Gorilla gorilla has been placed as homologous to chromosome 9 in man (see Chapter 3), and the C-band regions of the Y chromosome of the four species have been included in the diagram. Regions which are late replicating in these chromosomes are marked, and they correspond basically to regions of positive Q- G- and C-banding in all species. In some cases it has been found that the Q- G- or C-band regions which are late replicating in one species are the same as those which are late replicating in other species. A good example of this is found in chromosome 1 of man and its ape homologues. In HSA 1 there is a region which is late replicating but which is absent in the ape homologues (the secondary constriction). However, other regions of the human chromosome 1 do have homologies in the apes chromosome 1, i.e. regions p35, p33, p31, p21, q31, q41 and q43, and some of these can be seen to be late replicating in all species investigated. Findings of this kind are best observed in chromosomes where a good homology of

Q-/

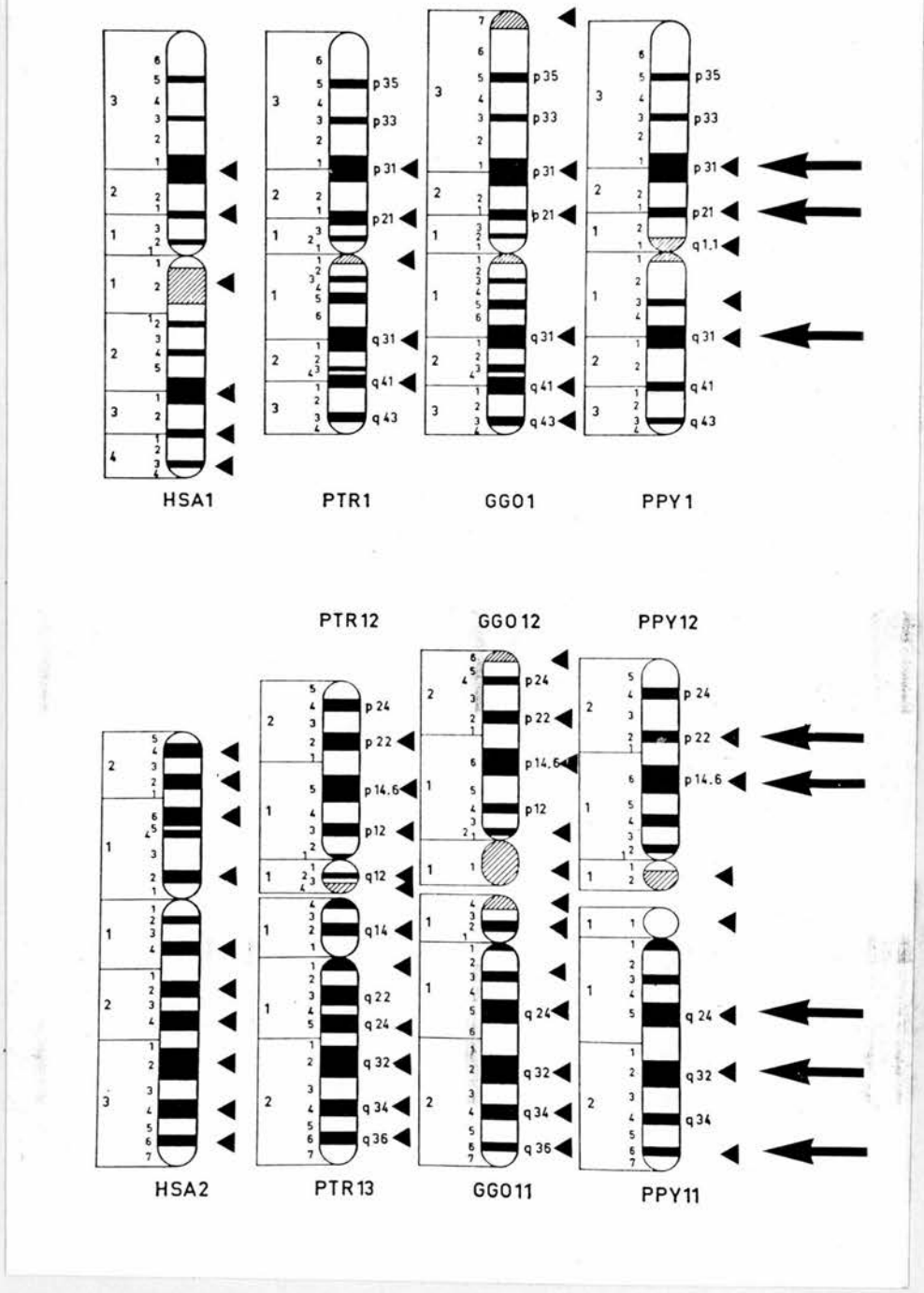


Fig.5.6(a) (See also 5.6(b), 5.6(c), 5.6(d), 5.6(e), 5.6(f), 5.6(g) and 5.6(h))

Comparison of the late DNA replicating regions of the chromosome of man and the three ape species studied. Short arrows point to positive G- and C- band regions in the diagrams. Long arrows point to similar (Homologous) chromosome bands which are late replicating in all species.

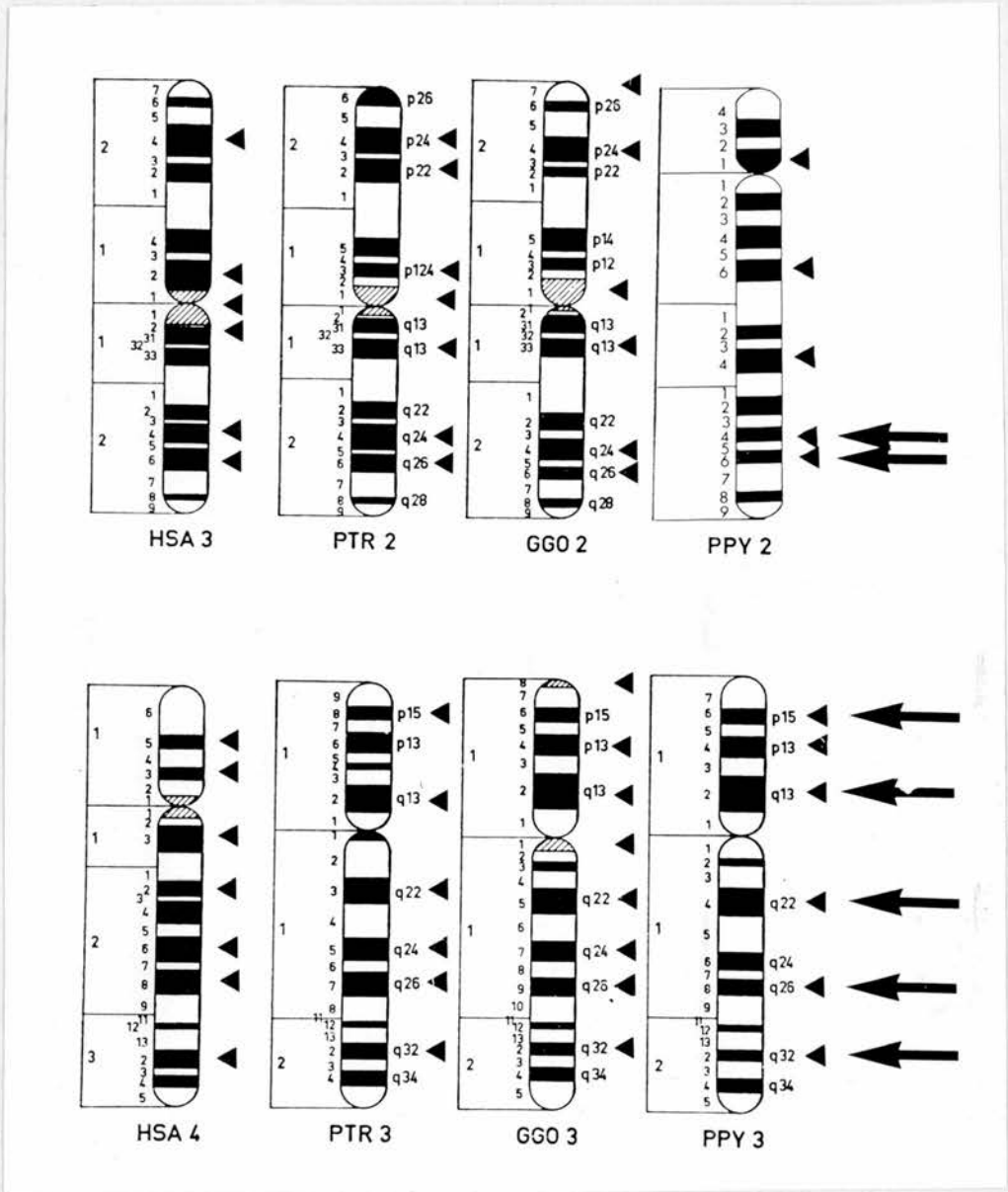


Fig.5.6(b)

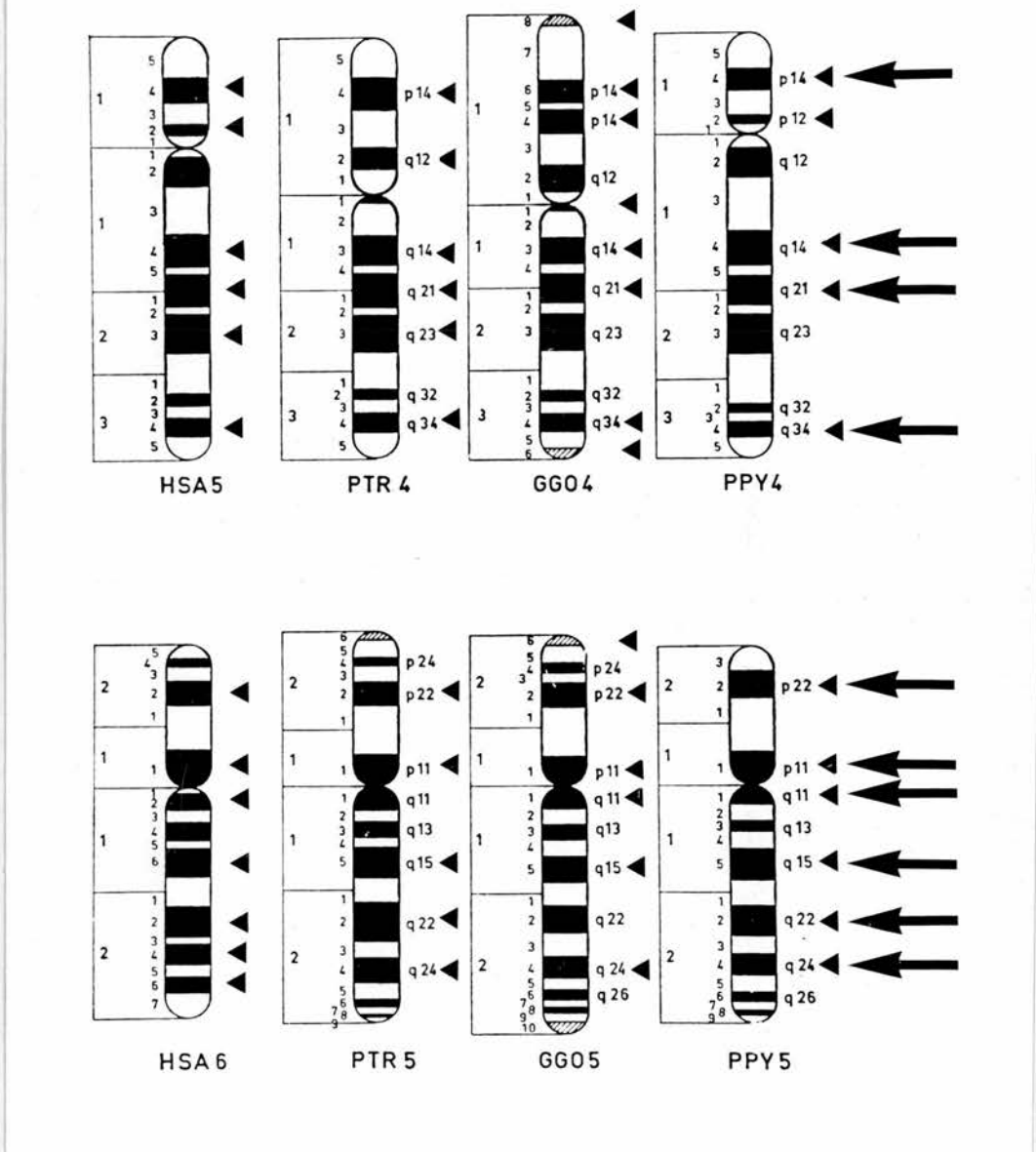


Fig.5.6(c)

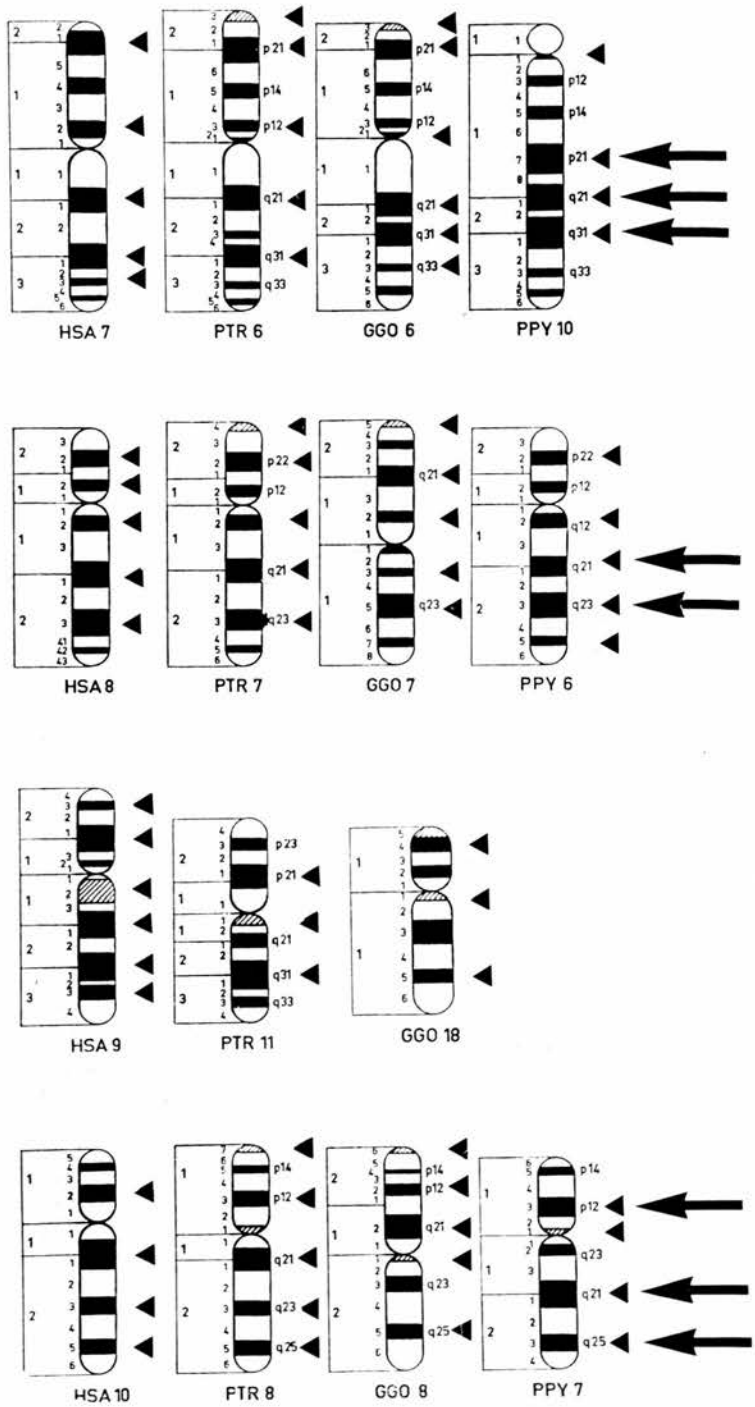
HSA 7
PTR 6
GGO 6
PPY 10
HSA 8
PTR 7
GGO 7
PPY 6
HSA 9
PTR 11
GGO 18
HSA 10
PTR 8
GGO 8
PPY 7

Fig.5.6.(d)

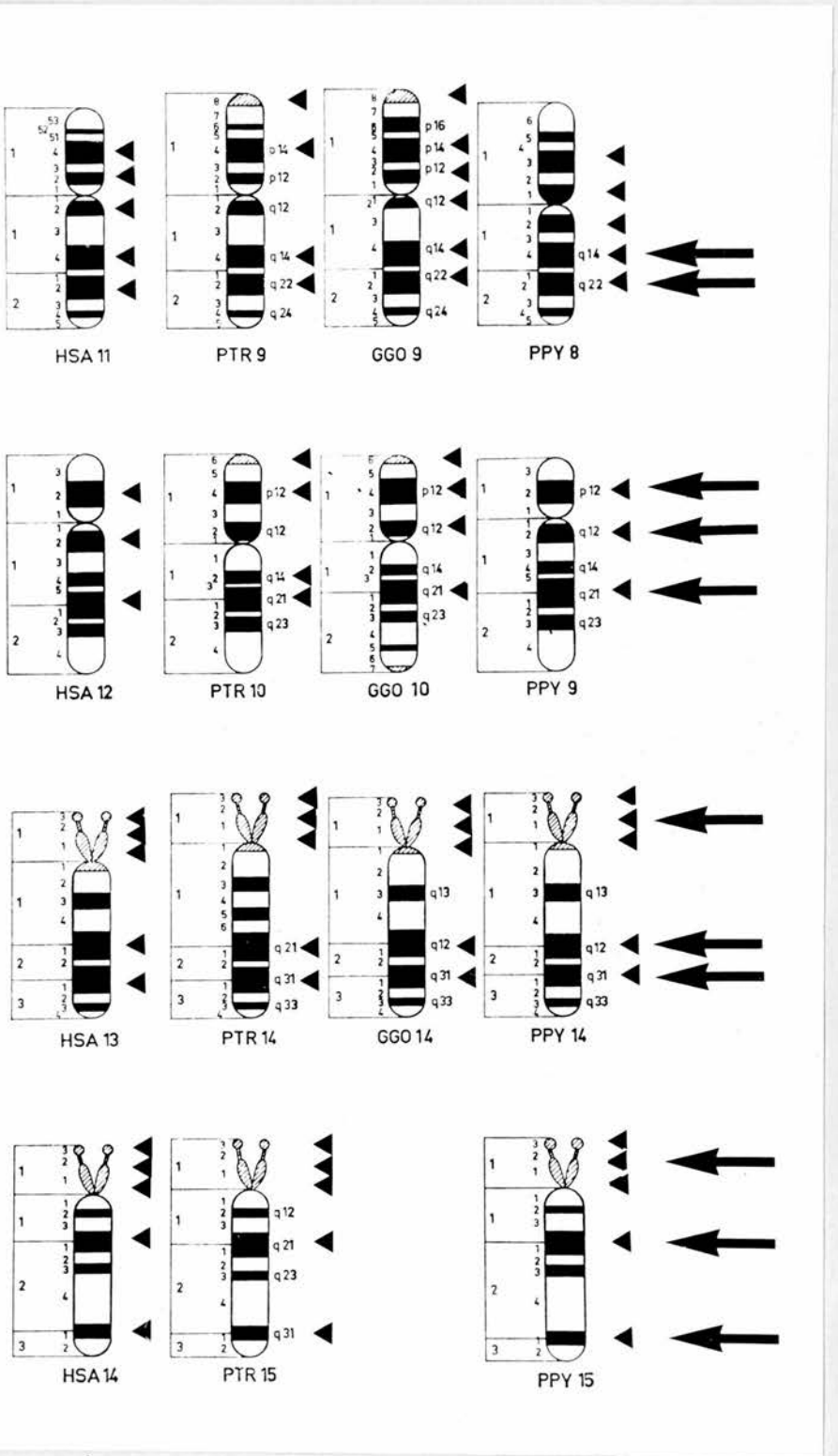
HSA 11
PTR 9
GGO 9
PPY 8
HSA 12
PTR 10
GGO 10
PPY 9
HSA 13
PTR 14
GGO 14
PPY 14
HSA 14
PTR 15
PPY 15

Fig.5.6(e)

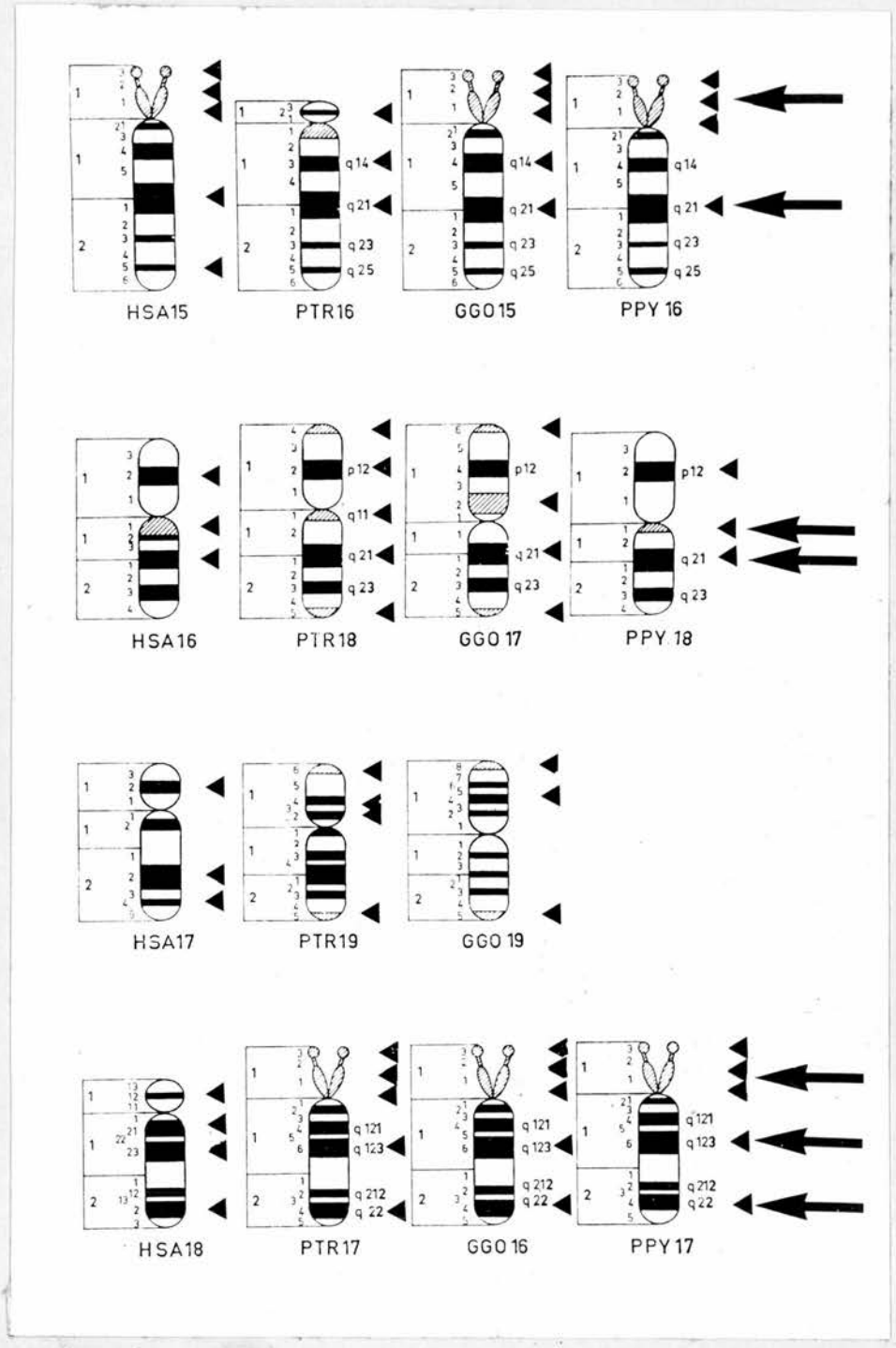
HSA15
PTR16
GGO15
PPY 16
HSA16
PTR18
GGO 17
PPY 18
HSA17
PTR19
GGO 19
HSA18
PTR17
GGO 16
PPY 17

Fig.5.6(f)

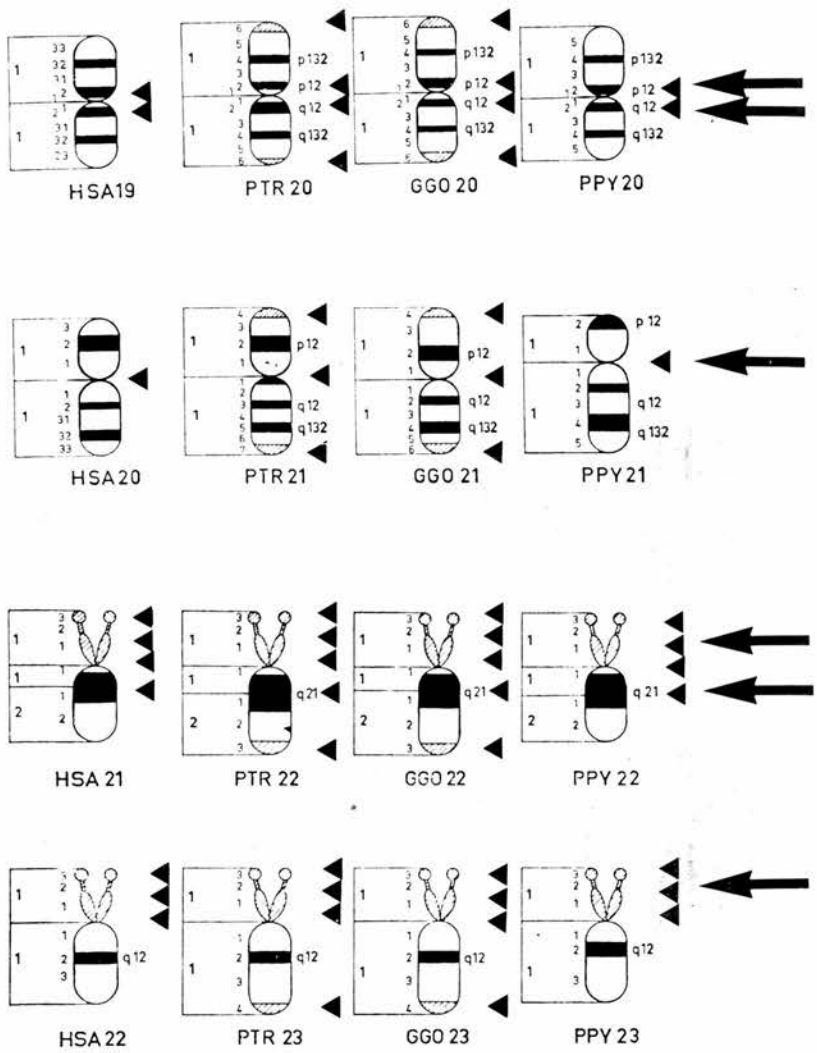
HSA19
PTR 20
GGO 20
PPY 20
p132
p12
q12
q132
HSA 20
PTR 21
GGO 21
PPY 21
p 12
q 12
q 132
HSA 21
PTR 22
GGO 22
PPY 22
q21
HSA 22
PTR 23
GGO 23
PPY 23
q12

Fig.5.6(g)

Q- and G-banding exists between different species, and where a chromosome band of a human chromosome can be precisely found in an ape chromosome (e.g. compare HSA 3 with PTR 2 and GGO 2). A remarkably conservative pattern of late replication was found in chromosome 13 of man and the ape homologues (PTR 14, GGO 14 and PPY 14). In man there are two clear regions of late DNA replication in the long arm of chromosome 13 which correspond to bands q21 and q31. These two regions are separated by a region of early DNA replication which corresponds to region q22, a region of negative G-banding (Fig.5.7). In PTR 14, GGO 14 and PPY 14 the same two regions are late replicating, thus showing an identical pattern of late replication as in man. This was clearly confirmed using cell cultures from a specimen of Gorilla gorilla in which a marker chromosome 14 was found [del(14)p31→p11:] , so that this chromosome could be unequivocally identified by its morphology. It is interesting to note that chromosome 14 of Pan troglodytes did not show a late replicating region at the site where the interstitial C-band is located and neither did chromosome 6 of this species where an interstitial C-band is found in the long arm. In situations in which chromosome homology exists between chromosomes of entirely different morphology, such as chromosome 2 in man and its ape homologues, there was also a good correspondence in the sites of late replication between homologous arms.

Q-band regions in human and ape chromosomes basically correspond to regions of positive G-banding with some minor exceptions (e.g. in the Y chromosome), and observations for G-band regions are therefore equally valid for Q-band regions. In man and the African apes some regions of the chromosome complement show brilliant fluorescence, but/

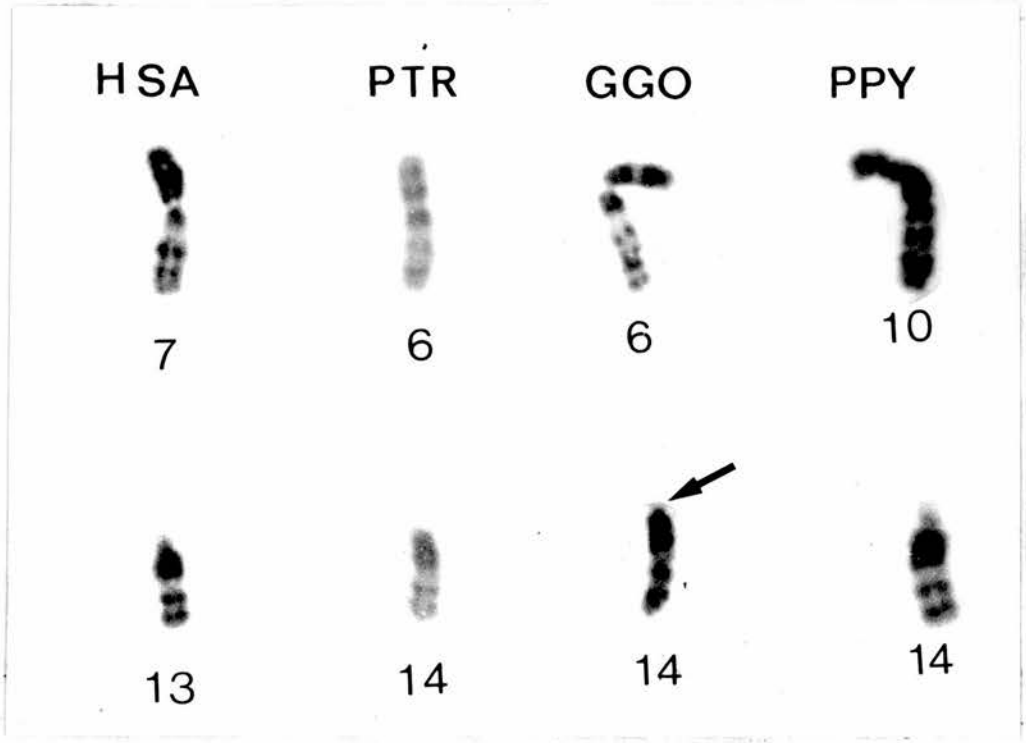


Fig.5.7. Conservation of late DNA replicating pattern in the Hominidae between chromosome 7 and 13 in man and the homologue chromosomes in the apes (B-pulsed cultures).

* Arrow indicates marker chromosome 14 in Gorilla gorilla.

but brilliant fluorescence is absent from all chromosomes in Pongo pygmaeus (see Chapter 4). All brilliant fluorescent regions correspond to regions of late DNA replication. In the Pongo pygmaeus, the chromosome regions homologous to those of brilliant fluorescence in other species were also found to be late replicating (e.g. the short arm region of chromosome 14 in Pongo pygmaeus which is homologous to the short arm region of chromosome 13 in man and 14 in Pan troglodytes and Gorilla gorilla). In man and Gorilla gorilla the brilliant fluorescent region of the Y chromosome was also late replicating.

Some late replicating regions of the chromosome complement of man and these three species of great ape coincided with regions of positive C-banding. Constitutive heterochromatic regions in these species of Hominidae have been located at the following sites: (i) centromeres of all chromosomes except for chromosome No.11 in Gorilla gorilla and No.9 in Pongo pygmaeus; (ii) at the secondary constriction of chromosome 1, 9 and 16 in man, and 17 and 18 in Gorilla gorilla; (iii) at the Y chromosome of all species; (iv) as interstitial C-bands in chromosome 6 and 14 in Pan troglodytes; (v) at the terminal (telomeric) regions of many chromosome arms in Pan troglodytes and Gorilla gorilla. These C-band regions appeared late replicating except for the interstitial C-band regions in Pan troglodytes. Terminal heterochromatic regions appeared, however, variable in their late replication pattern. It was common to find situations in which one terminal region was late replicating in one homologue whereas in the other homologue of the same individual this was not clearly evident. A comparison with the C-band karyotype of the same animal showed that terminal heterochromatic regions were present in both homologous chromosomes/

chromosomes, and the absence of the late replicating region in one chromosome could not therefore be due to the fact that the terminal region was absent from that chromosome.

Differences in the late replicating pattern of the X chromosomes was observed in female animals of these species, identical to those found in human female cells. In the first place, the X chromosome of the great apes is identical to the X chromosome of man in morphology and in Q-, G- and C-banding patterns, so that a straightforward comparison is possible. In T-pulsed cells one of the X chromosomes appeared more intensely stained than the other, and the regions of intense staining corresponded to regions of positive G-banding, probably bands p21, q21 and q23. The extreme tip of the long arm (band q27) of this chromosome was generally pale. The resolution at the band level was, however, poor in T-pulsed cells since the intensity of staining was very high. The other X chromosome showed the same pattern of late replication as the X chromosome in male cells, with a region of late replication at the short arm (p21) and another at the long arm (q21). The size of these two regions was clearly smaller compared to those of the more intensely stained X chromosome. From previous experiments in which cells had been grown in BUdr, but pulsed labelled with tritiated thymidine, we know that the X chromosome showing intense Giemsa staining corresponds to the late replicating X chromosome, whereas the other corresponds to the normally replicating X chromosome (Latt, 1974). A better understanding of the sites of DNA replication in the X chromosomes can be obtained from B-pulsed cultures. Regions of late replication appear as pale or negatively stained regions, and those of earlier replication as normally stained regions. As late replicating regions coincide with regions of positive

G-/

G-banding in T-pulsed cells, they coincide with regions of R-banding in B-pulsed cells. Thus, in B-pulsed cells, we found an X chromosome which appeared very pale compared with the rest of the chromosome complement (Fig.5.8 ), whereas the other X chromosome showed an identical replication pattern to that observed in the X chromosome of B-labelled male cells. The very pale X chromosome is obviously the late replicating X chromosome as has been demonstrated by Latt (1974) in cell cultures pulsed for the last five hours with tritiated BUdr. A close examination of the sequence of DNA replication in this chromosome shows that practically the entire X chromosome is pale (late replicating), except for two regions: one at the proximal segment of the short arm and another at the middle of the long arm (Fig.5.8 ). These regions coincide with regions of negative G-banding of the X chromosome (p11 and q22). It must be noticed that the terminal region of the long arm is also pale (late replicating) contrary to expectation, since the terminal region of the long arm of the late replicating X chromosome in most of the T-pulsed cells was pale (early replicating). However, there is evidence that this terminal region may sometimes be late replicating in the late replicating X chromosome as shown in Fig.5.8 (right side) where a pair of X chromosomes is shown from a human female T-pulsed culture in which the normally replicating X chromosome is compared with the late replicating ($X_L$) chromosome. In this latter, the terminal region of the long arm is intensely stained with Giemsa and the rest of the chromosome is pale.

(C) Discussion.

Our results show that in the human chromosome complement as in that of three species of great ape the sites of late DNA replication coincide/

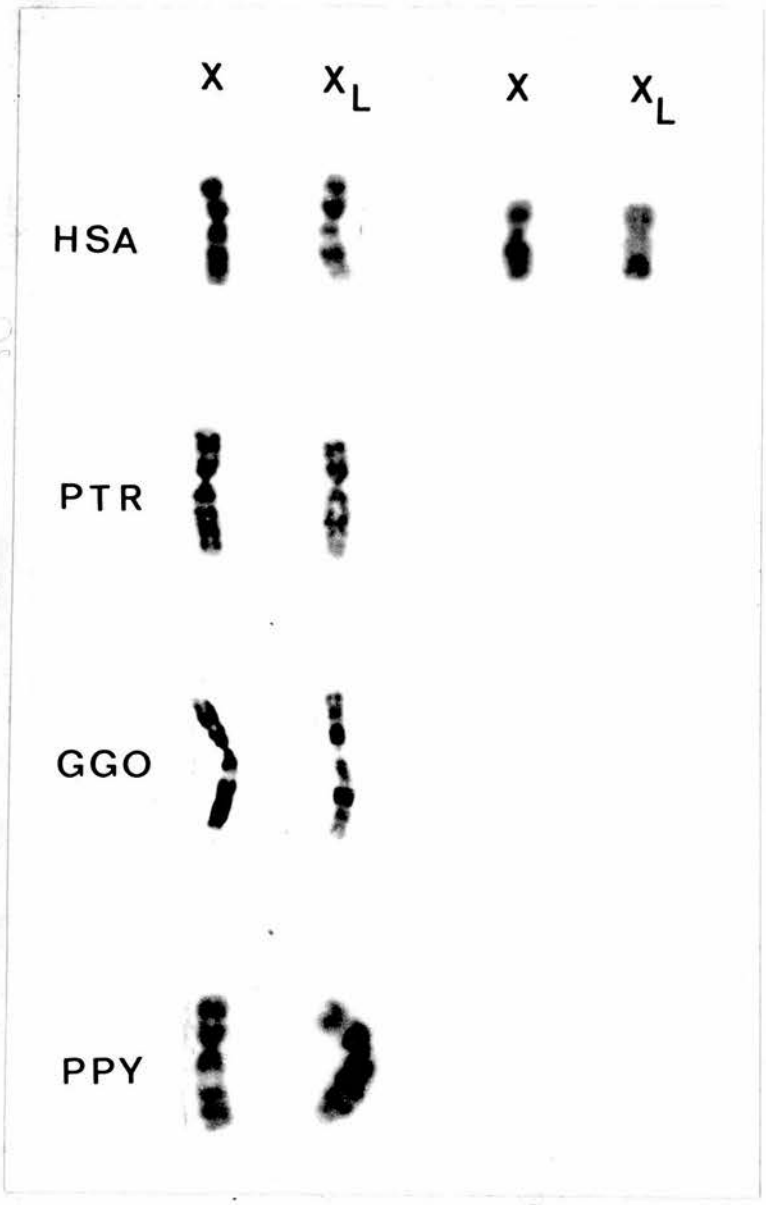


Fig.5.8. Differences between the timing of DNA replication of the X chromosomes in all the species studied. ($X_L$ denotes the late replicating X chromosome.)

coincide with regions of positive Q- G- and C-banding. They also point to the fact that the sequential pattern of DNA replication is similar in the different species studied.

These findings raise two important questions:-

(i) What is the relationship between sites of DNA replication and chromosome bands in these species and in general?

(ii) Why has a similar pattern of DNA replication been conserved in the Hominidae?

The first question is of interest since we know that in man heterochromatin is late replicating in relation to euchromatin (Lima de Faria and Jaworska, 1968). The observation that regions of positive Q- G- banding are late replicating in man (Ganner and Evans, 1971; Calderon and Schnedl, 1973; Latt, 1973, 1975; Epplen et al, 1975; Grzeschick et al, 1975), whereas regions of early DNA replication coincide with R-bands (Kim et al, 1975), suggests that Q- and G-bands are also heterochromatin. The definition of heterochromatin as initially stated by Heitz (1928) applied to those regions in the chromosome complement of organisms showing a preferential staining property, and which remain condensed throughout the entire cell cycle. More recently, the definition of heterochromatin has been widened and it includes two kinds of heterochromatin. One refers to the behaviour of one X chromosome which has become heterochromatic and genetically inactive in the somatic cells of mammalian species at some stage of embryonic development (Lyon, 1961), whilst remaining euchromatic in the germ cell line. This kind of heterochromatin has been designated "facultative". "Constitutive" heterochromatin, on the other hand, is that which according to Arrighi et al (1974) has the following properties:-

(1)/

(1) It remains condensed throughout the entire cell cycle except presumably during replication.

(2) It can be demonstrated by C-banding.

(3) It shows a definite and consistent pattern of distribution within the karyotype.

(4) It is genetically inert.

(5) It is usually late replicating in S-phase.

(6) It contains a large amount of repetitive DNA sequences (including satellite DNA).

It must be pointed out that this definition of "constitutive" heterochromatin is imprecise if we extend it to all organisms. In man, for example, the cytological demonstration of constitutive heterochromatin is evident with C-banding, but some regions of positive C-banding can also be demonstrated with G-banding, as the secondary constrictions of chromosome 1 and 16, especially when G-banding is obtained with proteolytic enzymatic digestion (Dutrillaux, 1975). In other regions of the human chromosome complement, however, G- and C-banding are mutually exclusive, as in the secondary constriction of chromosome 9 which is negatively G-banded and positively C-banded. In Pan troglodytes the interstitial C-band regions in chromosome 6 and 14 also correspond to positive Q- and G-band regions, whereas the terminal C-band regions of Pan troglodytes and Gorilla gorilla are negatively G-banded, but positively Q-banded. Thus, the strict differentiation between C- and G- (and sometimes Q-) band regions is not possible, and it might well be that G-band regions represent a different stage of heterochromatin than C-band regions, as has been proposed by Comings (1974).

The relation between chromosome banding and time of DNA replication/

replication is also conflicting. In the first place, similar chromosome banding patterns are obtained from cells of different tissues in man (e.g. lymphocytes and fibroblasts), but the pattern of late DNA replication in these two human cell types has been reported to be different (Slezinger and Prokofieva-Belgovskaya, 1968) as well as between lymphocytes and amniotic cells in culture (German and Aronian, 1971). Studies of late DNA replication in human lymphocytes has shown that some late replicating regions coincide with C-bands (e.g. the terminal region of the long arm of the Y chromosome), but others coincide with G-bands (e.g. bands q21 and q31 in chromosome 13) (Latt, 1973, 1975). In other organisms such as the Seba's fruit bat, Carollia perspicillata (Pathak et al, 1973), and in two species of kangaroo rat, Dipodomys merriani and Dipodomys panamintinus (Bostock and Christie, 1974, 1975) G- and Q-band regions have been found to start and complete their replication before other non Q- and non G-band regions. However, it has also been reported in the same species of kangaroo rat (Bostock and Christie, 1974, 1975), in the mouse, Mus musculus (Hsu and Arrighi, 1971; Hsu and Markvong, 1975), and in the indian muntjack, Muntiacus muntjack (Sharma and Dhaliwal, 1974), that some C-band regions start replicating earlier than other non C-band regions. Moreover, in the chinese hamster, Cricetulus griseus, it has been shown that G- and non G-band regions may replicate at different stages of the S-phase (Stubblefield, 1975). Thus, a straightforward relationship between chromosome bands and late DNA replicating sites is unlikely.

The sites of late DNA replication in human chromosomes do not necessarily coincide with the sites where satellite DNA sequences have been localized, and the same situation is valid for other species.

In/

In man, the Y chromosome distal long arm region is late replicating, it also corresponds to a heterochromatic region, and is the site where satellite DNA sequences have been localized (Evans et al, 1974b). However, many late replicating regions of the human chromosome complement do not contain detectable amounts of satellite DNA sequences (e.g. bands q21 and q23 in chromosome 13). Finally, some regions which are rich in satellite DNA sequences in Pan troglodytes (such as the proximal region of the long arm of chromosome No.6) do not appear to be late replicating, and the pattern of late replication of this chromosome is identical to that observed in chromosome No.7 of man and chromosome No.6 of Gorilla gorilla, where no major amounts of satellite DNA sequences have been detected (Seuanez et al, 1977b). In the chinese hamster the long arm of the X chromosome and the entire Y chromosome are heterochromatic and late replicating (Taylor, 1960; Stubblefield, 1975), but do not contain satellite DNA (Arrighi et al, 1974). A further comparison with other organisms shows that satellite DNA is not necessarily the last replicating fraction of the mammalian genome. In the mouse, for example, Tobia et al (1970) showed that 80% of the satellite DNA was synthesized after main band DNA, but there is also evidence that mouse satellite DNA terminates synthesis in the third quarter of S-phase (Bostock and Prescott, 1971). In one species of kangaroo rat (D. merriani) it was shown that one satellite fraction (MS) was early replicating as was GC rich main band DNA (Bostock and Christie, 1974). Thus, although replication of mammalian DNA in chromosomes is a co-ordinated process involving sequential replication of many heterogeneous DNA molecules it has no straightforward explanation in terms of the physical and biological properties of DNA molecules, or in terms of chromosome regions/

regions demonstrated by chromosome banding.

The reason why a similar pattern of late DNA replication is observed in the chromosome complement of man and the great apes has probably resulted from the conservation of the mechanisms of replication during phylogeny. There are few reports in the literature in which the late replicating patterns of the chromosomes of two or more phylogenetically related mammalian species showing closely similar karyotypes have been compared. The species of kangaroo rat studied by Bostock and Christie (1974, 1975) show remarkable differences in their karyotypes, and it is difficult to infer chromosome homologies between species, as we may do between man and the great apes.

Hsu and Markvong (1975) studied the late replicating pattern of three species and one subspecies of mouse (Mus musculus, Mus fulvidiventis, Mus dunni and Mus musculus mollosinus), all of which have a diploid chromosome number of 40, and show identical Q- and G-banding patterns. Differences between species and subspecies were due to the size of heterochromatic regions and of the sex chromosomes (Markvong et al, 1975). M.m.mollosinus showed heterochromatic regions which were unequal in size, Mus fulvidiventis was identical to Mus musculus except for a smaller Y chromosome, and Mus dunni showed heterochromatic short arms in the autosomes and the X chromosomes, and a large Y chromosome.

A comparison of the pattern of late replication showed that Mus musculus and Mus musculus mollosinus were identical; their centromeric regions were not the last to replicate in S-phase, and the Y chromosome finished replication before some autosomal regions. In Mus fulvidiventis, however, the centromeric regions were the last to/

to complete replication, but its smaller Y chromosome replicated earlier than the larger Y chromosome of Mus musculus. In Mus dunni the heterochromatic short arms of the autosomes were not late replicating, but the heterochromatic region of the X chromosome and the very long Y chromosome were late replicating. A comparison between these species has shown that not only the amount of constitutive heterochromatin has changed in the genus Mus, but also its sequential DNA replication, although the diploid chromosome number and the Q- and G-banding patterns have been conserved. This finding apparently contrasts with ours in the Hominidae, in which a constant pattern of sequential replication is observed between different species, although a greater variability is observed in chromosome number and morphology.

It would not be surprising if the difference observed within the genus Mus has resulted from the fact that species have diverged from each other at a very fast rate, as it usually occurs in the Rodentia in which reproductive performance is high, sexual maturity occurs early in life, litters are large, and animals live within restricted environments. Hsu and Markvong (1975) have postulated that these changes in replication patterns might result from a divergence in satellite DNA sequences at the heterochromatic regions of the genus Mus. Obviously in the Hominidae, the rate of evolutionary divergence must have been slower than in the Rodentia and this might explain the more conservative sequential pattern of replication in this subfamily.

The factors that control the sequential replication of chromosomes are unknown, but there is a standing controversy on whether each chromosome is capable of controlling its own sequential DNA/

DNA replication (by carrying the genetic information for initiation molecules which would act on its own replicons), or whether the control is mediated by the cytoplasm. Evidence in favour of cytoplasmic control is given by the fact that one X chromosome in female mammals becomes inactivated at some stage of embryonic development (Lyon, 1961), and the fact that patterns of late replication of chromosomes of cells of different origin are different in man (Slezinger and Prokofieva-Belgovskaya, 1968; German and Aronian, 1971). However, there is also evidence that each chromosome may control its own sequential replication in man, since in human-mouse hybrid cells the sequential pattern of replication of the human chromosomes is unchanged from what it was in the parental cell line. This suggests that the conservation of the pattern of sequential DNA replication does not require an intact human genome, and, since different chromosomes may be lost in the mouse-human hybrid cells, it is apparently independent of which chromosome had been eliminated (Lin and Davison, 1975). Graves (1975) has observed that hybrid cells of two species with different S-phase duration start S-phase synchronously, but each chromosome set has a similar S-phase period to the one it has in the parental cell line. A common pattern of chromosome replication between phylogenetically related species would result if the chromosomes of these species kept the genetic information for controlling their own sequential replication as in the common ancestor of the group. Thus, such conservation would be in a way comparable to the conservation of the original structural loci in the Hominidae as demonstrated by the report of the Baltimore Conference (1975).

(D) Important points of this Chapter. (Summary)

(1) The study of late DNA replication in the chromosomes of man, Pan troglodytes, Gorilla gorilla and Pongo Pygmaeus has shown that late replicating regions coincide with Q- G- and C-band regions.

(2) A comparison between chromosomes of different species has shown that homologous chromosomes show similar late replicating patterns.

(3) The sequential replication of the X chromosomes in female individuals was identical in all species.

(4) The relationship between late DNA replicating sites and chromosome bands has been discussed compared to the situation in other mammalian species.

(5) No straighforward relationship has been found between late replicating regions in the Hominidae and satellite rich regions.

(6) The factors involved in the regulation of DNA replication in chromosomes have been discussed.

# CHAPTER 6: CHROMOSOME BANDS IN MAN AND THE GREAT APES IN RELATION TO THE DISTRIBUTION OF DNA SEQUENCES HOMOLOGOUS TO THE FOUR HUMAN SATELLITE DNAs.

## (A) Introduction

The development of chromosome banding techniques has greatly increased our knowledge of chromosome substructure and organization. The chromosomes of three species of great ape (Pan troglodytes, Gorilla gorilla and Pongo pygmaeus) have been analysed in detail in the previous chapters and these studies have shown clear chromosome homologies between the three species, and between these species and man. However, although it has been reported that man and the great apes have 99% of their chromosome bands (G or R) in common (Dutrillaux, 1975), it must be remembered that banding reveals a degree of organization well above the molecular level, and that at the present time no banding technique gives unequivocal information about the nature of the underlying DNA (Summer, 1976). It is thus important to extend this comparison to the DNA level.

Two approaches are possible: (i) to see whether presumptive chromosome homologies correspond to homologies between linkage groups by assigning to chromosomes genes which are present in a few or single copies by the use of hybrid cell lines (see Baltimore Conference, 1975), and (ii) to study the location and distribution of highly repetitive DNA sequences which constitute a relatively high percentage of the total genome of man and eukaryotic organisms (Britten and Kohne, 1968). In man, for example, it has been estimated that repetitive DNA sequences account for at least 35-40% of the genome, half of which is highly repetitive and the other half/

half is moderately repetitive or intermediate (Arrighi and Saunders, 1973). Some of these highly repetitive sequences are apparently not transcribed, and their base composition differs from the average DNA base composition of the human genome, so that they may be separated from the main bulk of the DNA as distinctive satellite peaks when DNA is centrifuged to equilibrium in a heavy salt gradient. In man, four main satellite DNAs have been isolated using $Ag+$ and $Hg^{2+}$ ions in caesium sulfate gradients, and these have been designated I, II, III and IV (Corneo et al, 1967, 1970, 1971 and 1972). In Chapter 1 experiments which demonstrated the localization and distribution of these satellite sequences in the human chromosome complement were described. Also interspecific in situ hybridisation studies, using RNA complementary (cRNA) to a satellite fraction of one species applied to the denatured chromosome preparations of another species were mentioned. In this Chapter we report the results of experiments in which cRNA to the four human satellite DNAs was applied to denatured ape chromosomes (Gosden et al, 1977; Mitchell et al, 1977; Seuanez et al, 1977b) with emphasis on the relationship between interspecific chromosome homologies in the Hominidae and the distribution of hybridisation of the four human cRNAs.

(B) General observations on the distribution of repetitive DNA sequences homologous to the four human satellite DNAs in the chromosome complement of the great apes.

Of the three species of great ape studied, only one (Gorilla gorilla) showed detectable hybridisation with the four human cRNAs. cRNA II did not hybridise at all to the chromosomes of Pan troglodytes, and/

and cRNA IV showed significant amounts of hybridisation only at the Y chromosome in Pongo pygmaeus. cRNA III showed the highest amount of hybridisation as estimated by a higher grain count in the autoradiographs than any other cRNA and its distribution is shown in Figs. 6.1, 6.2 and 6.3. Fig.6.1 shows the chromosomes of Pan troglodytes in which each chromosome has been Q- and C-banded on the same preparation and compared to another member of the same chromosome pair which has been Q-banded and hybridised with cRNA III. Fig.6.2 and 6.3 show identical composite karyotypes for Gorilla gorilla and Pongo pygmaeus respectively. The pattern of distribution of hybridisation of the four cRNAs is shown in Table 6.1. The distribution of hybridisation of cRNA I in each species was basically similar to that of cRNA III, but the amount of hybridisation was lower as indicated by a lower grain count in the autoradiographic preparations (see Gosden et al, 1977). The distribution of hybridisation of cRNA II and IV in these species was, when detectable, also similar to that of cRNA III (see Table 6.1).

Only man and Gorilla gorilla have amplified the four highly repetitive sequences (I, II, III and IV), so that in that respect these two species resemble each other more closely than any other of the four studied. However, the fact that man and Gorilla gorilla share four highly repetitive sequences in common does not mean that a greater degree of phyletic affinity exists, but there is ample evidence that such similarity has arisen by parallelism. There are two sets of evidence to suggest that the amplification of these sequences has occurred after speciation and that this event occurred independently in each species (Gosden et al, 1977; Seuanez/

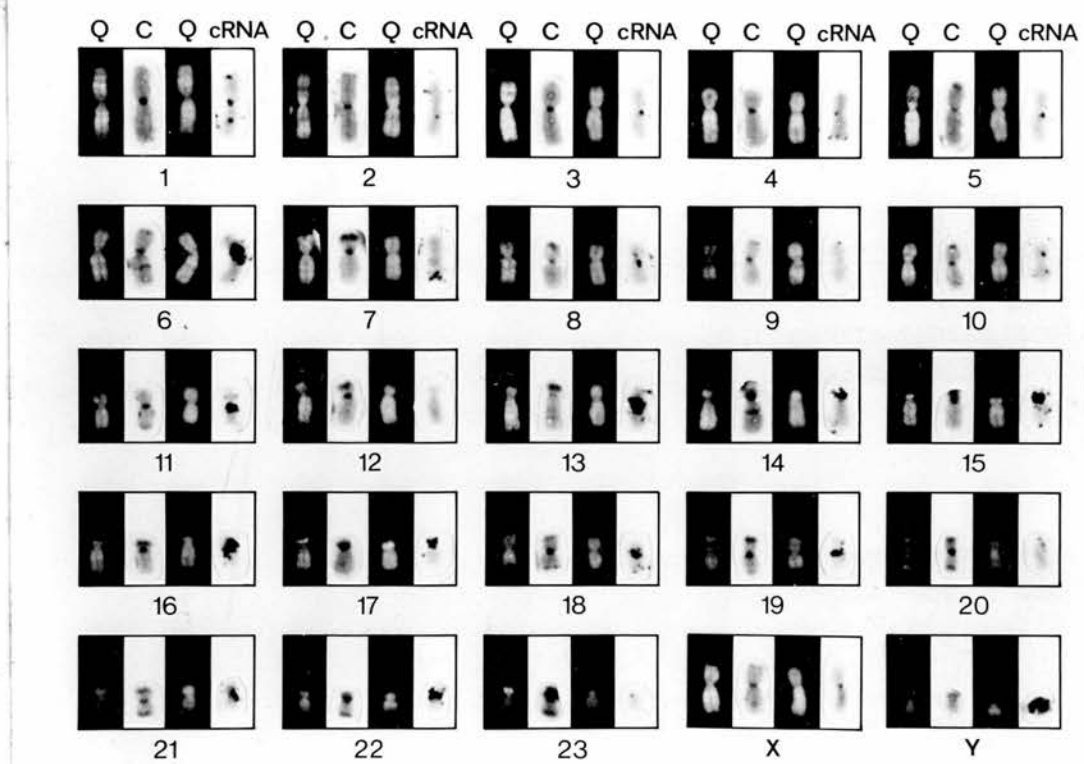


Fig.6.1. Q- and C-band patterns and Q and *in situ* hybridisation patterns with cRNA III of *Pan troglodytes*. The picture was composed with chromosomes from two different cells.

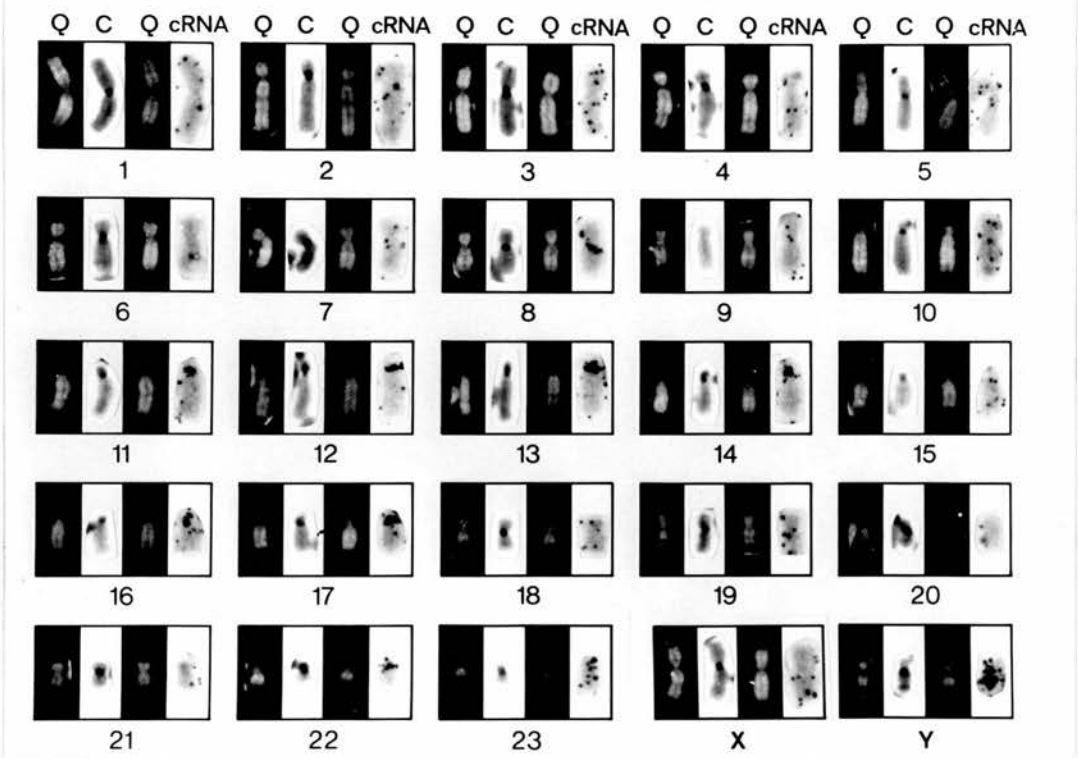


Fig.6.3. Q- and C-band patterns and Q and in situ hybridisation patterns with cRNA III of Pongo pygmaeus. The picture was composed with chromosomes from two different cells.

TABLE 6.1.

IN SITU HYBRIDISATION OF HUMAN cRNA I, II, III AND IV WITH HOMINOID METAPHASE CHROMOSOMES (a and b)

| Homo | I | II | III | IV | Pan | I | III | IV | Gorilla | I | II | III | IV | Pongo | I | II | III |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | 1 | - | + | - | 1 | - | + | + | + | 1 | - | - | - |
| 2p | - | - | - | - | 12 | - | - | - | 12 | + | + | + | + | 12 | + | + | + |
| 2q | - | - | - | - | 13 | + | + | + | 11 | - | - | - | - | 11 | + | + | + |
| 3 | - | - | - | - | 2 | - | - | - | 2 | - | - | - | - | 2 | - | - | - |
| 4 | - | - | - | - | 3 | - | - | - | 3 | - | - | - | - | 3 | - | - | - |
| 5 | + | - | + | + | 4 | - | - | - | 4 | + | + | + | - | 4 | - | - | - |
| 6 | - | - | - | - | 5 | - | - | - | 5 | - | - | - | - | 5 | - | - | - |
| 7 | + | + | + | + | 6 | + | + | + | 6 | - | - | - | - | 10 | - | - | - |
| 8 | - | - | - | - | 7 | - | - | - | 7 | - | - | - | - | 6 | - | - | - |
| 9 | + | + | + | + | 11 | + | + | + | 18 | + | + | + | + | - | - | - | - |
| 10 | - | - | - | + | 8 | - | - | - | 8 | - | - | - | - | 7 | - | + | - |
| 11 | - | - | - | - | 9 | - | - | - | 9 | - | - | - | - | 8 | + | - | - |
| 12 | + | - | - | - | 10 | - | - | - | 10 | - | - | - | - | 9 | + | - | - |
| 13 | + | + | + | + | 14 | + | + | + | 14 | + | + | + | + | 14 | + | + | + |
| 14 | + | + | + | + | 15 | + | + | + | - | - | - | - | - | 15 | + | + | + |
| 15 | + | + | + | + | 16 | + | + | + | 15 | + | + | + | + | 16 | + | + | + |
| 16 | - | + | - | - | 18 | + | + | - | 17 | + | + | + | + | 18 | - | - | - |
| 17 | - | + | - | + | 19 | + | + | + | 19 | - | - | - | - | - | - | - | - |
| 18 | - | - | - | - | 17 | + | + | + | 16 | + | + | + | + | 17 | + | + | + |
| 19 | - | - | - | - | 20 | - | - | - | 20 | - | - | - | - | 20 | - | - | - |
| 20 | + | + | + | + | 21 | + | + | + | 21 | - | - | - | - | 21 | - | - | - |
| 21 | + | + | + | + | 22 | + | + | + | 22 | - | - | - | - | 22 | + | + | + |
| 22 | + | + | + | + | 23 | + | + | + | 23 | + | + | + | + | 23 | + | + | + |
| X | - | - | - | - | X | - | - | - | X | - | - | - | - | X | - | - | - |
| Y | (+) | + | + | + | Y | + | + | + | Y | + | + | + | + | Y | + | + | |
| | | | | | | | | | 13 | + | + | + | + | 13 | + | + | + |
| | | | | | | | | | | | | | | 19 | - | - | - |

+ indicates detectable hybridisation above grain background.

a = Gosden et al (1977). b = Mitchell et al (1977).

Seuanez et al, 1977b). In the first place, the fact that Pongo pygmaeus binds cRNA II and Pan troglodytes does not, suggests that this sequence was present in the ancestor, since Pongo split from the common trunk of the Hominidae before the separation of man, Pan and Gorilla. The most logical explanation is that this sequence has been amplified in Pongo, and in Gorilla and man, but not in Pan. On the other hand, satellite IV was amplified to a lesser degree in Pongo than in the other three species. The alternative process, amplification previous to speciation is less likely. This would imply that one sequence was significantly reduced or lost in all chromosomes of Pan (satellite II), and another was significantly reduced in Pongo (satellite IV). The second evidence comes from a comparison of which kind of sequence is detected in homologous chromosomes when the karyotypes of the Hominidae are compared following the criteria of the Paris Conference (1971); supplement (1975). Chromosome 16 in man, for example, binds only cRNA II at the region of its secondary constriction. Its homologue in Pan troglodytes (PTR 18) does not bind cRNA II since none of the chromosomes in this species binds cRNA II, but binds cRNA I, and cRNA III. The corresponding homologue in Gorilla gorilla (GGO 17) binds significant amounts of cRNA I, II, III and IV, and in Pongo pygmaeus the homologous chromosome (PPY 18) only binds significant amounts of cRNA III (Gosden et al, 1977; Mitchell et al, 1977). Chromosome homologies as revealed by banding generally correspond with data on gene assignment (Baltimore Conference, 1975). However, they contain different kinds of repetitive sequences, a fact that again suggests that the process of amplification occurred independently after speciation.

(C)/

(C) Chromosome banding patterns in relation to sites of hybridisation.

(i) Q-band regions.

In man brilliant fluorescent regions may contain satellite DNAs (e.g. the Y chromosome). However other brilliant fluorescent regions (e.g. the centromere of chromosome 3 and 4) contain no detectable satellite DNA. Regions stained negatively with quinacrine (e.g. the secondary constrictions of chromosomes 1, 9 and 16) contain large amounts of satellite DNA. In Pan some brilliant fluorescent regions are rich in satellite DNAs (e.g. PTR 14, see Fig.1). However, the very pale staining Y chromosome and the pale centromeric region of PTR 16 are also major sites of hybridisation with cRNA I, III and IV.

In Gorilla some brilliant fluorescent regions (e.g. GGO 12 and the Y chromosome; see Fig.2) are major sites of hybridisation with the four cRNAs, but other brilliant fluorescent regions are not (GGO 3 and 22). Pale staining regions such as the short arm of the Y chromosome are sites of hybridisation, as are the negative staining secondary constrictions of GGO 17 and 18. In Pongo, where there is no brilliant fluorescence there are many sites of hybridisation of cRNAs I, II and III in the autosomes, and with the four cRNAs in the Y chromosome.

(ii) G-band regions.

Excluding the regions of constitutive heterochromatin (e.g. region cen→ q12 in HSA 16 or region cen →q13 in HSA 9) there are few regions in the Hominidae exclusively characterized by G-banding in which satellite DNAs have been localized. One of these can be found/

found in chromosome 5 in man (HSA 5) at the subcentromeric region (q12) where there is a minor site of hybridisation with cRNA III and IV. The ape homologous chromosomes (PTR 4, GGO 4 and PPY 4) carry a similar G-band region to that found in HSA 5. In PPY 4, the G-band pattern is identical to HSA 5 so that a positive G-band region is found (q12) which corresponds to that of HSA 5. In PTR 4 and GGO 4, both of which are more metacentric chromosomes, this region has been located at the proximal region of the short arm (p12), probably as a result of a pericentric inversion. When these regions are compared in relation to their content of satellite DNA sequences, those in PTR 4 and PPY 4 show no detectable hybridisation, whereas in GGO 4 this region is a site of hybridisation with the four cRNAs.

Chromsome 7 in man has a very minor site of hybridisation with cRNA IV at its centromere. Its homologue in Pan troglodytes (PTR 6) is very similar to HSA 7 (Fig.6.1) but this chromosome is a major site of hybridisation with cRNA I,III and IV. The site of hybridisation extends to the subcentromeric regions probably (cen→q21) and covers a region which is negatively G-banded (positively R-banded) similar to region cen→ q21 in HSA 7. This result shows that two cytologically equivalent regions contain entirely different amounts of satellite DNA. In PTR 6 there is only a small C-band at the centromere (Fig.6.1) . though the overall amount of satellite DNA is higher than that found at the secondary constriction of chromosome 9 in man, a region with a large C-band.

(iii) C-band regions.

When constitutive heterochromatin regions, as demonstrated by C-banding/

C-banding, are compared with the sites of hybridisation with cRNA I, II, III and IV in the Hominidae three kinds of results are obtained:-

iii.a. C-band regions showing detectable amounts of hybridisation.

In man, the heterochromatic segment of the Y chromosome, of the secondary constriction region of chromosome 1, 9 and 16, and the centromeric region of the acrocentric chromosomes contain satellite DNA, although each kind of sequence may be present in different amounts as estimated by grain counts. In two species of great ape, Gorilla and Pongo the large heterochromatic segments of the Y chromosome are major sites of hybridisation. In Gorilla, hybridisation with cRNA I, III and IV show grains covering the whole of Y chromosome though the grain count was higher at the long arm, but cRNA II hybridised at the region of the long arm which is brilliant with fluorescence. In Pongo there are two definite areas of hybridisation in the Y chromosome which coincide with the positive C-band regions. The higher amount of hybridisation was observed at the larger C-band region, at the centromeric region of this chromosome.

The secondary constriction of chromosome 17 and 18 in Gorilla were sites of hybridisation with the four cRNAs. All positively C-banded centromeric regions of the acrocentric chromosomes in Pan, Gorilla and Pongo were also sites of hybridisation (Fig.6.1, 6.2 and 6.3) except PTR 12, GGO 22 and PPY 10 (GGO 11 shows no hybridisation, but is not included in this list since its centromeric region is not positively C-banded).

iii.b. Constitutive heterochromatin segments in the Hominidae which do not show detectable amounts of highly repeated DNA.

In man, most of the positively C-banded regions of the large metacentric chromosomes do not show detectable amounts of satellite DNA (Gosden et al, 1975). Most large metacentric chromosomes in the complement of the great apes did not hybridise any of the four cRNAs at the centromeric region. The interstitial heterochromatic regions of PTR 6 and 14, and all the terminal heterochromatic segments of the chromosome complement of Pan and Gorilla did not bind significant amounts of any cRNA, nor did the acrocentric chromosomes mentioned above (PTR 12, GGO 22, and PPY 10) at the centromeric region.

iii.c. Highly repeated DNA sequences detected in regions which cannot be demonstrated as constitutive heterochromatin by C-banding techniques.

The Y chromosome of Pan only C-bands positively at its very small short arm, but the whole chromosome is obliterated with grains after hybridisation with cRNA I, III and IV, and a major site of hybridisation is also found in chromosome 6 of Pan troglodytes. It is interesting that the Y chromosome and chromosome 6 in Pan show higher amounts of total hybridisation (higher grain counts for all cRNAs hybridised) than the human Y chromosome and chromosome 9 in man, respectively. However, in man, the satellite rich regions of these two chromosomes can be demonstrated as constitutive heterochromatin by C-banding.

(D) Discussion.

(i) Q-band regions.

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In the great apes as in man the intensity of fluorescence seems to be unrelated to the presence or the amount of any of these highly repeated DNA sequences. In GGO Y there is an apparent coincidence in the location of satellite II and brilliant fluorescence (see Gosden et al, 1977). However, chromosome 1 and 16 in man have regions rich in this kind of DNA sequence but neither of them shows brilliant fluorescence (Jones et al, 1971). Satellite II is undetectable in Pan troglodytes although there is brilliant fluorescence in the chromosome complement of this species; satellite II is detectable in Pongo pygmaeus, but there is no brilliant fluorescence at all in the chromosomes of this species. In relation to the phylogeny of the chromosomes of the Hominidae, it is remarkable that brilliant fluorescence is present at the same regions in many homologues of the chromosomes of man and the great apes of Africa, a finding that suggests that these species have a common origin (Pearson, 1973). It would appear that man and the African apes (the Homininae) differ from the Ponginae (Pongo pygmaeus) in having fully amplified one repeated DNA sequence (satellite IV) and by showing brilliant fluorescence. It is, however, highly improbable that the amplification of this kind of satellite DNA is in some way related to the appearance of brilliant fluorescence in the chromosomes of the Homininae for the following reasons:-

(a) There is no brilliant fluorescence in PPY Y and PTR Y though these chromosomes contain DNA satellite-IV sequences as do GGO Y and HSA Y, although the latter pair fluoresce brilliantly.

(b) There appears to be no relationship between the location or the amount of satellite IV and the intensity of fluorescence in many chromosomes/

chromosomes of man, Pan troglodytes and Gorilla gorilla. For example, the brilliant fluorescent regions of HSA 3, HSA 4, GGO 3 and GGO 22 do not show hybridisation with cRNA IV (or with any satellite cRNA; Gosden et al, 1977; Mitchell et al, 1977). On the other hand, the subcentromeric region of the Y chromosome in GGO Y shows a relatively high amount of this kind of DNA sequence, although this proximal region is not brilliantly fluorescent.

(ii) G-band regions.

The mechanism involved in G-banding is still obscure, although the suggestion that it is simply related to DNA composition is improbable, since G-banding depends on the nature of associated proteins and their differential distribution of free sulphydril and disulphide bonds (Summer, 1974). Our present studies show that G-banding is unrelated to the satellite DNA content in the chromosomes of the Hominidae for the following reasons:-

(a) Highly repetitive DNAs may be present at either positively or negatively G-banded regions;

(b) Two chromosome regions of identical G-banding pattern may show totally different kinds and amounts of highly repetitive DNAs.

G- (or R-) banding, however, show chromosome homologies between the Hominidae (see Paris Conference, 1971; supplement 1975), and most of these homologies have been confirmed by gene assignment (Baltimore Conference, 1975) suggesting that G-banding demonstrates homologous linkage groups in the Hominidae. As G- (or R-) banding reflect a degree of chromosome organization determined by the nature of the associated proteins, they must depend, indirectly, on the genes codeing for such structural proteins present on each homologous/

homologous chromosome pair. Similar banding patterns between chromosomes of phylogenetically related species such as man and the great apes would result if these genes were conserved in newly formed species. Thus, a chromosome pair would keep the original structural genes, including those for chromosomal proteins involved in the mechanisms of banding that were present in the common ancestor of the group. This is reflected by the fact that man and the great apes have 99% of their bands in common (Dutrillaux, 1975), although the position of these bands differs between species as a consequence of chromosome rearrangement.

(iii) C-band regions.

The comparison between constitutive heterochromatin as demonstrated by C-banding and the sites of hybridisation in the chromosomes of the Hominidae produced three different results:-

iii.a. Positive C-banding - Positive hybridisation.

iii.b. Positive C-banding - Undectectable hybridisation.

iii.c. Negative C-banding - Large amount of hybridisation.

The first finding (iii.a.) is consistent with some properties of constitutive heterochromatin (Arrighi et al, 1974): that it can be demonstrated by C-banding, that it has a consistent pattern and distribution in the karyotype, and that it contains large amounts of highly repeated DNA including satellite. The fact that some heterochromatic segments of the chromosome complement of the great apes are able to bind cRNA to the four human satellites must be evaluated carefully. A positive hybridisation shows that homologous sequences to those of man have been amplified in the genome of the great apes. The degree of homology is impossible to define/

define with this kind of experiment, but the conditions under which *in situ* hybridisation was done were stringent, and this must avoid high amounts of mismatching in the formation of the stable hybrid (see Gosden *et al*, 1977). On the other hand, the fact that cRNA II does not hybridise to any of the chromosomes of *Pan* gives good evidence that cross reaction does not exist, at least between satellite II sequences and other sequences, although cRNA II hybridised at some of the same sites as cRNA I, III and IV in man, *Gorilla* and *Pongo*.

iii.b. *Positive C-banding; undetectable amounts of hybridisation.*

Three different possibilities can be considered to explain this finding:-

(1) Highly repeated sequences may exist at a region which is positively C-banded, but in amounts which are below those detectable by *in situ* hybridisation.

(2) Other kinds of highly repeated sequences may exist at a positively C-banded region. Such sequences may not cross react with any of the four cRNAs used. This may explain why the four cRNAs to the human satellite DNAs do not hybridise to regions such as the interstitial heterochromatin of PTR 6 and PTR 14, or to any of the terminal heterochromatic segments in the chromosome complement of *Pan* and *Gorilla*. This implies that *Pan* and *Gorilla* have amplified other sequences which may be either not present at all, or present but not amplified, in the human genome.

(3) Positively C-banded regions may not contain highly repeated sequences in significant amounts, as in the sex chromosomes of the chinese hamster (Arrighi *et al*, 1974). C-banding would then be a consequence/

consequence of DNA packaging, only indirectly of DNA composition.

iii.c. Positive Hybridisation at negatively C-banded regions.

Discarding the possibility that emulsion grains might coalesce from one region to another during prolonged exposure time, the detection of positive hybridisation in regions which cannot be demonstrated as constitutive heterochromatin by C-banding is a finding of interest. This finding reaffirms those of Arrighi et al (1974) in that C-banding is not unequivocally related to the nature of DNA. This is coincident with the generally held view on C-banding because C-bands in different organisms have been found to contain entirely different kinds and amounts of DNA. For example, in the mouse, C-banding reflects AT rich satellite DNA sequences (Pardue and Gall, 1970), in the oxen, G-C rich satellite DNA sequences (Kurnit et al, 1973), whilst in Microtus agrestis only moderately repeated DNA sequences are detected (Arrighi et al, 1970). Finally, in the Chinese hamster there are only insignificant amounts of highly repeated DNA in the genome, although the chromosome complement of this species shows significant C-banded regions (Arrighi et al, 1974).

The appearance of the positively C-banded secondary constrictions in some of the metacentric chromosomes of the Hominidae is an intriguing finding. If we compare, for example, the long arm of chromosome 1 in man we find a secondary constriction which is absent in the ape homologues. Can we say that the appearance of such a constriction in the lineage leading to man was achieved by amplification of highly repeated DNAs? This is highly improbable for two main reasons:-

(a)

(a) that some amplification is already present in PTR 1 and GGO 1 at the same homologous arm, and

(b) that the degree of amplification, even if very high, does not imply the formation of a secondary constriction or a large C-banded region (e.g. compare HSA 7 and PTR 6; HSA Y and PTR Y). We must then conclude that regions demonstrated by C-banding reflect sites of DNA organization, and that this organization probably involves protein molecules (see Comings, 1974).

(iv) G-11 regions and satellite III rich regions.

Other techniques have been used to demonstrate selectively some regions of the constitutive heterochromatin of the human chromosome complement, such as the G-11 technique (Bobrow et al, 1972; Gagne and Laberge,1972). These techniques have also been used to study the chromosome complement of Pan troglodytes (Bobrow and Madan, 1973; Lejeune et al, 1973), Gorilla gorilla (Bobrow et al, 1972; Dutrillaux et al, 1973; Pearson, 1973), and Pongo pygmaeus (Dutrillaux et al, 1975c). Each of these reports used different criteria of nomenclature and of presumptive homologies between human and ape chromosomes, but a comparative table of results can be obtained if their observations are adapted to the standard criterion of the Paris Conference (1971); supplement (1975) (Table 6.2). In this Table we have also added the sites of hybridisation with cRNA III on the chromosome of these species as presented by Mitchell et al (1977), since it has been claimed (see Bobrow et al, 1972; Bobrow and Madan, 1973; Pearson, 1973; Jones, 1976) that the G-11 technique selectively demonstrated satellite III rich regions in the chromosome complement of man and the great apes.

From/

TABLE 6.2

COMPARISON OF THE G-11 STAINING AND IN SITU HYBRIDISATION OF HUMAN cRNA III TO HOMINOID METAPHASE CHROMOSOMES.

| Homo | | | Pan troglodytes | | | | Gorilla gorilla | | | Pongo pygmaeus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chr. | cRNA III | G-11[a] | Chr. | cRNA III | G-11[a] | G-11[b] | Chr. | cRNA III | G-11[c] | Chr. | cRNA III | G-11[d] |
| 1 | q | q | 1 | p | p | cen | 1 | p | cen | 1 | - | cen? |
| 2p | - | - | 12 | - | - | q | 12 | p | p | 12 | p | p |
| 2q | - | - | 13 | q | q | - | 11 | - | - | 11 | p | p |
| 3 | - | - | 2 | - | - | - | 2 | - | - | 2 | - | - |
| 4 | - | q | 3 | - | - | - | 3 | - | - | 3 | - | - |
| 5 | q | - | 4 | - | - | - | 4 | q | - | 4 | - | - |
| 6 | - | - | 5 | - | - | - | 5 | - | - | 5 | - | - |
| 7 | - | q | 6 | q | p | p | 6 | - | p | 10 | - | cen |
| 8 | - | - | 7 | - | - | - | 7 | - | - | 6 | - | - |
| 9 | q | q | 11 | q | q | q | 18 | q | q | - | | |
| 10 | - | q | 8 | - | - | - | 8 | - | p | 7 | - | q |
| 11 | - | - | 9 | - | - | - | 9 | - | - | 8 | - | - |
| 12 | - | - | 10 | - | - | - | 10 | - | - | 9 | - | - |
| 13 | p | p | 14 | p | p | p | 14 | p | p | 14 | p | p |
| 14 | p | p | 15 | p | p | p | - | - | - | 15 | p | p |
| 15 | p | p | 16 | p | p | p | 15 | p | p | 16 | p | p |
| 16 | - | - | 18 | p | p | p | 17 | p | p | 18 | - | - |
| 17 | - | p | 19 | q | q | cen | 19 | - | - | - | | |
| 18 | - | - | 17 | p | - | p | 16 | p | p | 17 | p | p |
| 19 | - | - | 20 | - | - | - | 20 | - | q | 20 | - | - |
| 20 | cen | p | 21 | q | q | q | 21 | - | - | 21 | - | - |
| 21 | p | p | 22 | p | p | p | 22 | - | p | 22 | p | p |
| 22 | p | p | 23 | p | p | p | 23 | p | p | 23 | p | p |
| X | - | - | X | - | - | - | X | - | - | X | - | - |
| Y | q | q | Y | p+q | p | ? | Y | p+q | q[e] | Y | q | ? |
| | | | | | | | 13 | p | p | 13 | p | p |
| | | | | | | | | | | 19 | - | p |

(p = short arm of chromosome)

(q = long arm of chromosome)

(cen = centromere)

(? = staining not clearly visible)

(a) Bobrow and Madan (1973)

(b) Lejeune et al (1973)

(c) Dutrillaux et al (1973)

(d) Dutrillaux et al (1975c)

(e) Bobrow et al(1972)

From data in Table 6.2 it can be seen that in man, the location of G-11 regions coincides with satellite III rich regions except for Nos.4, 7,10 and 17 in which there are no detectable amounts of satellite III. In Pan troglodytes both reports on the distribution of G-11 regions are coincident (Bobrow and Madan, 1973; Lejeune et al, 1973), except that in the report of Lejeune et al (1973) it is the proximal region of the long arm of chromosome 12 (HSA 2p) that shows positive G-11 staining, and in that of Bobrow et al (1973) it is the proximal region of chromosome 13 (HSA 2q) that is stained. A comparison with the sites of in situ hybridisation with cRNA III shows that there is good correlation between these sites and the G-11 stained regions, except for chromosome 12 in which there is no satellite III. The Y chromosome is stained only in its very short arm, but it is completely obliterated when hybridised with cRNA III. The pattern of G-11 banding in Gorilla gorilla has been shown in photographs by Bobrow et al (1972)and Pearson (1973) but with the exception of the Y, the chromosomes were not identified. Dutrillaux et al, (1973) studied the Giemsa 11 pattern of this species in a female animal in which all chromosomes were identified, and their findings are presented in Table 6.2. It is important to note, however, that the results shown by Bobrow et al (1972) and Pearson (1973) differ from those of Dutrillaux et al (1973). In the former reports there are three large metacentric chromosomes with their entire short arm region intensely stained with Giemsa, and this was interpreted as one autosomal pair and probably the X chromosome (Bobrow et al, 1972), whereas in the latter report the size of G-11 regions was small and never involved whole arms of metacentric chromosomes or the X chromosome. There is no explanation of this apparent/

apparent difference and the fact that G-11 was obtained in chromosomes by different procedures is an unlikely explanation. A comparison between the sites of G-11 staining shown by Dutrillaux et al (1973) and the sites of hybridisation with cRNA III are generally coincident except for Nos. 4, 6, 8, 20 and 22. A comparison of results shown by Bobrow et al (1972) and Pearson (1973) coincide for the Y chromosome but hybridisation with cRNA III never covered whole arms of large metacentric chromosomes or of the X chromosome.

The G-11 pattern of Pongo pygmaeus presented by Dutrillaux et al (1975) generally agrees except for three chromosome regions. The centromeric region of chromosome 10, the subcebtromeric region of chromosome 7 and the region above the centromere of chromosome 19 all show a positive G-11 region, but no satellite III. This comparison suggests that although the sites of G-11 staining and the satellite III rich regions generally coincide, there is no one to one specificity between both events, since it is possible to find regions of positive staining with no satellite III, and vice versa. One of the ways to clarify this point completely would be to do G-11 staining and in situ hybridisation on the chromosome preparations obtained from the same individual, either human or ape, so that we can discount the possibility that variations in the amount and location of satellite DNA III between individuals may produce misleading comparisons. However, a comparison between the G-11 patterns of Gorilla gorilla as shown by Bobrow et al (1972) with the later presented by Dutrillaux et al (1973) makes it unlikely that such differences in staining might correspond to differences in the location (or amount) of satellite III between the/

the animals studied. It rather points to the fact that the technique is less accurate in showing a constant pattern, at least in this species, and that its demonstration of satellite III rich regions is also less precise than with *in situ* hybridisation techniques.

(E) Summary

(1) Homologous sequences to the human satellite DNAs (I, II, III and IV) have been localised in the chromosome complement of three species of great ape with *in situ* hybridisation techniques.

(2) Only man and *Gorilla gorilla* have amplified the four DNA sequences (I, II, III and IV). *Pan troglodytes* showed no detectable hybridisation with human cRNA II, and *Pongo pygmaeus* only hybridised human cRNA IV to the Y chromosome.

(3) Positive hybridisation of cRNA II in *Pongo pygmaeus* (but not in *Pan troglodytes*), and the distribution of hybridisation of the four cRNAs between homologous chromosomes of different species indicated that amplification of these four DNA sequences has occurred independently after speciation.

(4) Q- and G-bands in these species were unrelated to the presence of the amount of these four DNA sequences.

(5) There was no one to one specificite between regions of constitutive heterochromatin and regions of hybridisation with the four human cRNAs in these species.

(6) A comparison between G-11 stained regions and satellite DNA rich regions was not entirely coincident, indicating that the G-11 technique is less precise in showing satellite III regions than *in situ* hybridisation.

## CHAPTER 7: CHROMOSOME EVOLUTION IN MAN AND THE GREAT APES.

The evolution of man continues to be a subject of intense investigation, and a comparison between the chromosomes of the Hominidae allows a reconstruction of what might have been the phylogeny of the chromosomes of man and his closest living relatives. Three questions arise in relation to this problem:

(A) What are the presumptive mechanisms by which the chromosomes of man and the great apes have diverged?

(B) How important was this process in speciation?

(C) Which of the four species of great ape is (a) more closely similar to man in its chromosome constitution, and (b) phylogenetically closer to man as demonstrated by cytotaxonomy?

(A) A direct comparison of the karyotypes of the Hominidae shows that similarities exist between different species, as demonstrated by chromosomes with similar morphology and banding pattern. Inter-specific chromosomal similarity is the criterion used in the Paris Conference (1971); supplement (1975) to recognize presumptive chromosome homology between species. Different degrees of inter-specific similarity may be found in the Hominidae:-

(i) between chromosomes with identical morphology and G- (or R-) banding pattern;

(ii) between chromosomes of very similar (but not identical) morphology, but showing practically identical G- (or R-) banding pattern. Morphological change can be due to (a) the amount or the location of constitutive heterochromatin; (b) the amount of brilliant fluorescent material; (c) the amount of highly repeated DNA sequences;

(iii)/

(iii) between chromosomes of different morphology and G- (or R-) banding pattern, which, however, can be derived one from the other by chromosome rearrangement;

(iv) between chromosomes with a similar morphology with G- (or R-) banding patterns not strictly coincident which cannot be derived from each other by chromosome rearrangement.

Finally, some chromosomes may show completely different morphology and G- (or R-) banding pattern to those of other species. Complete absence of interspecific similarity implies that no presumptive interspecific homologous chromosomes can be inferred.

Thus, presumptive homology between chromosomes of different species is based on similarity, but homology may be inferred from different degrees of similarity.

(i) Chromosomes with identical morphology and G- (or R-) banding pattern in all species.

Four pairs of autosomes and the X chromosome are practically identical in all species. Autosomes No. 6, 19, 20 and 21 in man have identical homologues in the great apes. The comparison is, however, restricted to G- (or R-) band regions since heterochromatic regions (e.g. the terminal C-bands in Pan and Gorilla), or the brilliant fluorescent regions (which are absent in Pongo pygmaeus) are not taken into consideration. Otherwise, only the X chromosome would be identical in all species.

(ii) Chromosomes with very similar morphology in all species.

This group of chromosomes is listed in Table 7.1. Chromosome

1/

TABLE 7.1

CHROMOSOMES WITH SIMILAR (BUT NOT IDENTICAL) MORPHOLOGY SHOWING PRACTICALLY IDENTICAL G-BANDING PATTERN IN ALL SPECIES

| Homo | Pan troglodytes | Pan paniscus | Gorilla gorilla | Pongo pygmaeus |
|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 |
| 13 | 14 | 14 | 14 | 14 |
| 15 | 16 | 16 | 15 | 16 |
| 16 | 18 | 18 | 17 | 18 |
| 18 | 17 | 17 | 16 | 17 |
| 22 | 23 | 23 | 23 | 23 |

1 in man has a heterochromatic secondary constriction which is absent in the ape homologues. A high degree of similarity exists, however, between its non C-band regions and the non C-band regions in the great ape chromosome 1. Chromosome 13 in man has a similar homologue in all other species except in Pan troglodytes where there is a heterochromatic region at the long arm (see Chapter 2). Chromosome 15 in man corresponds to an acrocentric chromosome in the genus Pan (PTR 16 and PPA 16) from which that part of the short arm including the stalk and the satellite (the 18S and 28S rDNA sequences) have been deleted (Henderson et al, 1976a). Otherwise it is similar to its homologue in each other species. Chromosome 16 in man has a secondary constriction which is absent in the genus Pan and in Pongo pygmaeus but is present in Gorilla gorilla (GGO 17). However, in GGO 17 the secondary constriction is found in the short arm of the chromosome, probably resulting from a pericentric inversion. Since the inversion involved a heterochromatic region, we consider this chromosome to be similar to chromosome 16 in man, as in human populations we would consider a chromosome 9 with an inverted secondary constriction to be similar to a normal chromosome 9. Chromosome 18 in man has a short arm with no satellite stalk or terminal satellite. In the apes, on the other hand, terminal satellites and satellite stalks are present. It would appear that deletion has eliminated, in man, regions which in the ape homologues are sites of brilliant fluorescence (in PTR 17, PPA 17, GGO 16), and/or contain satellite DNA sequences (in PTR 17, GGO 16 and PPY 17; see Chapter 6), and/or 18S and 28S rDNA cistrons (in PTR 17, PPA 17 and PPY 17; see Henderson et al, 1976a). A comparison between chromosome 22 in man and its ape homologues shows that although the/

the long arms are identical in all species, the homologue in Pan paniscus (chromosome 23) is metacentric, having a short arm which is totally heterochromatic and showing a terminal region of brilliant fluorescence. A comparison between many chromosomes of Pan troglodytes, Pan paniscus and Gorilla gorilla with their homologues in man and Pongo pygmaeus shows that in the former three species there is a large number of heterochromatic regions at the telomeres of chromosome arms which are absent in the latter two species (Chapters 2 and 3). All these changes, however, have not affected the euchromatic regions of the chromosomes. The change which has occurred from one species to another somewhat resembles the polymorphic changes which one normally observes within human populations or when comparing relatively large groups of apes. Thus, we can consider that 10 autosomal pairs and the X chromosome have been conserved in the Hominidae with minimal change. However, if individual pairs of species are compared higher numbers of chromosomes can be seen to have been conserved as follows: (numbers in parenthesis correspond to the human chromosomes to which the ape chromosomes are homologous)

Man-Pan troglodytes and Man-Pan paniscus: 17 autosomes + the X chromosome. (1, 3, 6, 7, 8, 9, 10, 11, 13, 14, 15, 16, 18, 19, 20, 21, 22)

Man-Gorilla gorilla: 14 autosomes + the X chromosome. (1, 3, 6, 7, 9, 11, 13, 15, 16, 18, 19, 20, 21, 22.)

Man-Pongo pygmaeus: 14 autosomes + the X chromosome. (1, 5, 6, 8, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22.)

Pan-troglodytes-Pan paniscus: 22 autosomes + the X chromosome. (1,/

(1, 2p, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22.)

Pan troglodytes-Gorilla gorilla and Pan paniscus-Gorilla gorilla: 17 autosomes + the X chromosome. (1, 2p, 3, 4, 6, 7, 9, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22.)

Pan troglodytes-Pongo pygmaeus and Pan paniscus-Pongo pygmaeus: 14 autosomes + the X chromosome. (1, 2p, 4, 6, 8, 13, 14, 15, 16, 18, 19, 20, 21, 22.)

Gorilla gorilla-Pongo pygmaeus: 14 autosomes + the X chromosome. (1, 2p, 2q, 4, 6, 13,15,16,18,19, 20, 21, 22), and one pair of chromosomes in Gorilla gorilla (GGO 13) and another in Pongo pygmaeus (PPY 13) which are very similar to each other, but neither of which is homologous to any chromosome of man, Pan troglodytes or Pan paniscus.

Thus, a comparison between pairs of species shows that a very large number of virtually unchanged chromosomes (22 autosomes plus the X chromosome) have been maintained within the genus Pan, an intermediate number (17 autosomes plus the X chromosome) between Homo and Pan, and Gorilla and Pan, and the lowest number (14 autosomes plus the X chromosome) between Homo and Gorilla, Gorilla and Pongo, and Pongo and Pan.

(iii) Homologous chromosomes between species which can be derived from each other by chromosome rearrangement of G- (or R-) band regions.

The following rearrangements can be postulated to derive one chromosome from another:-

(i)/

(i) Pericentric inversion.

(ii) Paracentric inversion.

(iii) Telomeric fusion.

The pericentric inversion is the most common type of rearrangement to explain differences between homologous chromosomes of different morphology. The following pericentric inversions have been proposed (Fig.7.1), and they have probably taken place in the direction indicated, based on the principle of parsimony.

PPY 2 (Bornean chromosome) → PPY 2 (Sumatran chromosome)

PPY 2 (Bornean chromosome) → PTR 2, PPA 2, GGO 2, HSA 3

PTR 3, PPA 3, GGO 3, PPY 3 → HSA 4

HSA 5, PPY 4 → PTR 4, PPA 4, GGO 4

HSA 7, PTR 6, PPA 6, GGO 6, ⇄ PPY 10

HSA 8, PTR 7, PPA 7, PPY 6 → GGO 7

HSA 10, PTR 8, PPA 8 → GGO 8

HSA 12, PPY 9 → PTR 10, PPA 10, GGO 10

A paracentric inversion could explain the difference between HSA 10, PTR 8, PPA 8 → PPY 7.

Telomeric fusions may account for the differences in chromosome number between the apes and man. Good homology exists for example between the G-band pattern of HSA 2p and PTR 12, and HSA 2q and PTR 13. Other changes have probably occurred between various ape species, for example:-

(a) PPY 12p (carrying satellite DNA and rDNA cistrons but not brilliant fluorescence) → GGO 12p (carrying satellite DNA but not rDNA cistrons; has brilliant fluorescence) → partial deletion of the/

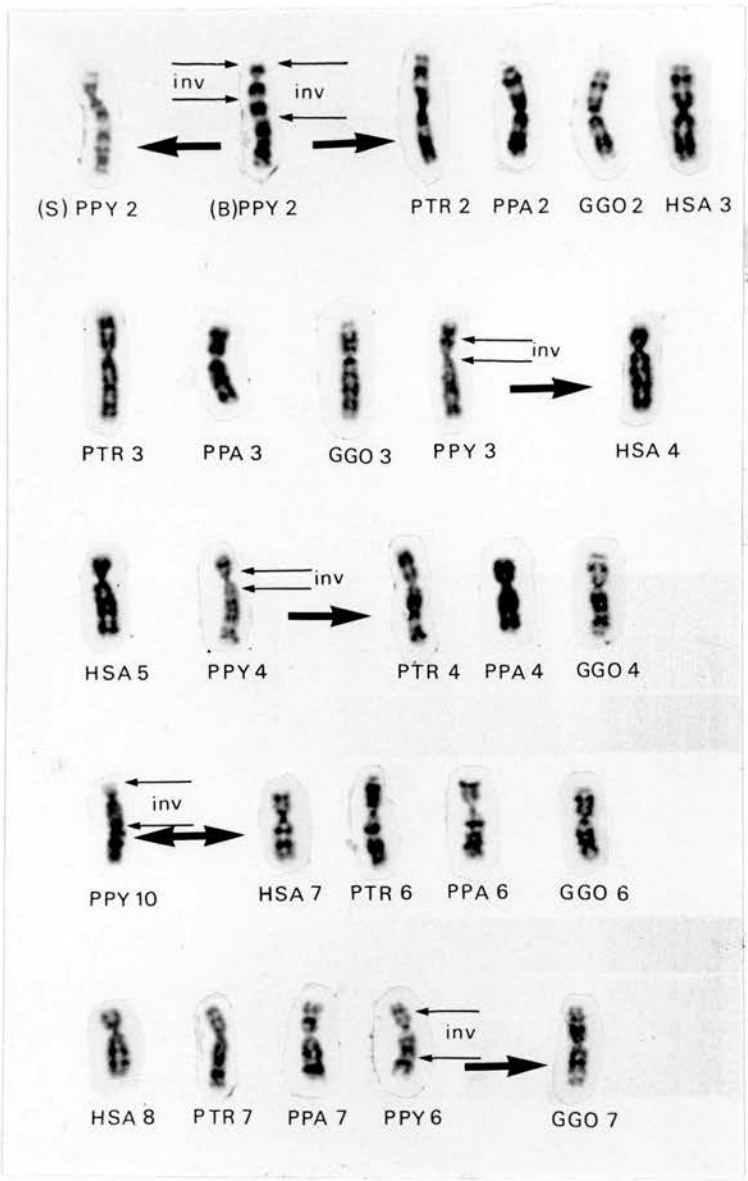



Fig.7.1. (a and b) Inversions through which chromosomes of one species could be derived from those of another. Small arrows indicate chromosome breaks and large arrows point to the probable direction in which chromosome rearrangement took place.

the short arm → PTR 12p (no satellite DNA; no brilliant fluorescence), and PPA 12p (satellite DNA unknown; no brilliant fluorescence).

(b) PPY 11p (carrying satellite DNA and rDNA cistrons but not brilliant fluorescence) → partial deletion of the short arm → GGO 11p (no satellite DNA, no rDNA cistrons and no brilliant fluorescence) → new G-band region → PTR 13p (satellite DNA at centromeric region but no brilliant fluorescence) → new G-band region → PPA 13p (satellite DNA unknown; no brilliant fluorescence).

(iv) Homologous chromosomes with a similar morphology but with G-banding pattern which does not coincide nor which can be derived by chromosome rearrangement.

On morphological grounds and on evidence provided by studies on gene mapping, chromosome 17 in man appears to be homologous to PTR 19, PPA 19 and GGO 19. Of these chromosomes, PTR 19 and PPA 19 are identical, but the G-band patterns of HSA 17, PTR 19 (or PPA 19) and GGO 19 do not coincide precisely, and the ape chromosomes are somewhat more metacentric than HSA 17. Thus, although these chromosomes might carry identical genes (Baltimore Conference, 1975) their banding patterns cannot be matched as precisely as other chromosomes in the complement. Reorganization of chromatin has probably occurred to account for the existing differences, but it cannot be deduced by a comparison of the G-band patterns of the group. A similar situation is found between chromosome 13q in Pan troglodytes and its homologue arm in Gorilla gorilla (GGO 11q) which shows a slightly different G- (or R-) banding pattern and they cannot be derived from each other by chromosome rearrangement. Another example of this situation is found at the distal region of the short arm of chromosome

4/

4 in Gorilla gorilla compared to its homologues in any other species. The short arm of chromosome 13 in Pan paniscus also shows an additional G-band region which is absent in chromosome 13 of Pan troglodytes, and cannot be derived from it. An identical situation is found in chromosome 8 of Pongo pygmaeus when compared to its homologues (HSA 11, PTR 9, PPA 9 and GGO 9).

(v) Chromosomes having no homologous counterpart in any other species.

The Paris Conference (1971); supplement (1975) considers GGO 13, GGO 18, PPY 13 and PPY 19 to be in this category. However, we have considered GGO 18 as homologous to HSA 9, PTR 11 and PPA 11 (see Chapter 3), and GGO 13 and PPY 13 as homologous to each other. This leaves only one chromosome in Pongo pygmaeus which has no homologue in any of the other species (PPY 19).

The Y chromosome.

Finally, it is interesting to comment on the Y chromosome of the Hominidae which is clearly different between species. The changes in this chromosome are mainly due to heterochromatic material and/or brilliant fluorescence. The direction in which events have occurred is difficult to envisage, but a comparison with other primates may be illuminating. In Hylobates lar and Hylobates concolor (gibbon) (Tantravahi et al, 1975, Dutrillaux et al, 1975a) the Y chromosome is very small, and the rule seems to be that in most primates the Y chromosome is also very small, as reported by Chiarelli (1967), except in one species of old world monkey (Cercopithecus diana. Within the Hominidae, the smallest Y chromosome is that of Pan troglodytes, and this might correspond to the ancestral/

ancestral Y chromosome of the group. In relation to the intensity of fluorescence, only the Y chromosome of man and the gorilla show brilliant fluorescence, a finding which suggests that this is a relatively new event in the evolution of the primates.

Evolution of karyotypes by chromosome rearrangement.

(a) Pericentric inversion.

A comparison between different species of Hominidae shows that the most common type of chromosome rearrangement within the group are pericentric inversions (Turleau et al, 1972; Bobrow and Madan, 1973; de Grouchy et al, 1973). It is interesting to note that pericentric inversions have been reported in Pongo pygmaeus (Turleau et al, 1975; Seuanez et al, 1976a,b), in the gibbon, Hylobates moloch, (Tantravahi et al, 1975), and in the squirrel monkey, Saimiri sciureus, (Ma et al, 1974; Lau et al, 1976), a species in which inversions distinguish subspecies from various geographical locations. Inversions have been postulated as a mechanism of chromosome evolution in the Primates (Egozcue, 1969), the Felidae (Roubin et al, 1973), and in some species of Rattus (Yoshida, 1973).

(b) Translocations and centric fission.

Non Robertsonian translocations are rarely a mechanism of chromosome evolution in animals (White, 1973), but one translocation has been suggested to have occurred in the lineage leading to Gorilla gorilla between the short arm of chromosome 4 and a small chromosome of the complement (Dutrillaux, 1975). The possibility that fission might have occurred in the Hominidae is evident when analysing/

analysing the karyotypes of two species of gibbon (Hylobates lar and Hylobates moloch) which have a diploid number of 44, and an entire complement of metacentric chromosomes. A comparison of the karyotypes with those of man and the great apes show that homologies exist between some arms of the metacentric gibbon chromosomes and some acrocentric chromosomes of the Hominidae (Tantravahi et al, 1975). This might be an indication that some acrocentric chromosomes of man and the great apes have arisen by fission of metacentric chromosomes. Further evidence for chromosome fission in the primates comes from studies of comparative gene mapping in man, the baboon (Papio papio) and the African green monkey (Cercopithecus aethiopes) (Finaz et al, 1977). Chromosome 1 in the baboon is similar to chromosome 1 in man except that it has no secondary constriction, and its long arm is inverted in relation to the G-band pattern of HSA 1q. In the African green monkey, each arm of chromosome 1 in the baboon has a corresponding acrocentric homologous chromosome. Two gene markers present in chromosome 1 of man were also found in chromosome 1 of the baboon. Two genes carried by the short arm of chromosome 1 in man were assigned to the acrocentric chromosome of the African green monkey homologous to the short arm of chromosome 1 in the baboon (and to the short arm of chromosome 1 in man). One gene carried by the long arm of chromosome 1 in man was assigned to the acrocentric chromosome in the African green monkey homologous to the long arm of chromosome 1 in the baboon (and to the long arm of chromosome 1 in man, but inverted). Thus, the emergence of a metacentric chromosome 1 in the baboon and in the Hominoidea could be explained by two alternative mechanisms both of which require an inversion: one which requires two fusions of acrocentric chromosomes which took place independently in/

in the lineage leading to the baboon and in the lineage leading to the Hominoidea. The other, more economical, only needs one fission in the line leading to the African green monkey. The principle of parsimony indicates that the hypothesis involving one fission might be the most likely event in the phylogeny of this chromosome. Centric fissions have been reported to occur in man as a result of which telocentric chromosomes resulted from a metacentric (Sinha et al, 1972; Hansen, 1975; Dallapicolla et al, 1976), and in one case a telocentric marker was transmitted to the offspring (Sinha et al, 1972). In the primates, Egozcue (1971) reported a centric fission which produced a stable long telocentric chromosome and an unstable short telocentric chromosome in the Entellus monkey, Presbystis entellus. In mammals, centric fissions have been reported; by Fredga and Bergstrom (1970) in the root vole, Microtus oeconomus.

(c) Telomeric fusion.

The mechanism by which two chromosomes fused by their telomeres to form a large metacentric chromosome (HSA 2) in the line leading to man necessarily involves a mechanism of centromere inactivation to account for the existence of one functional centromere in chromosome 2 in man. There are reports in the literature of metacentric chromosomes in man in which two distinguishable centromeric regions have been visualized with C-banding and this has resulted from fusion of two acrocentric chromosomes, each retaining its own centromeric region (Hsu et al, 1973). The same phenomenon has been observed in the formation of X isochromosomes (Niebuhr and Skovby, 1977). The behaviour of these chromosomes in mitosis suggests, however, that one centromere has become inactivated. In the case of chromosome 2 in man/

man, the centromeric region of the ape chromosome which is homologous to the long arm of HSA 2 must not only have been inactivated, but transformed into a euchromatic region. Only one heterochromatic region is found in chromosome 2 in man, and it corresponds to the centromeric region of the ape chromosome homologous for the short arm of the human chromosome 2. It is interesting to note that the centromere region of the HSA 2q homologue in Gorilla gorilla (GGO 11) is not heterochromatic, and does not hybridise with cRNA to any of the four human satellite DNAs. In Pan troglodytes, however, where a better homology exists between the banding pattern of HSA 2q and PTR 13q, the centromere region of PTR 13 is one of the major sites of hybridisation with cRNA I,III and IV, and this region is heterochromatic. Thus, it is impossible to find a phylogenetic sequence which would fit both G-band homology and "euchromatinization" of the centromeric region for the long arm of chromosome 2 in man. It must also be pointed out that "euchromatinization" of the centromeric region, though it has occurred, can not explain centromere inactivation by itself. A good example is the absence of constitutive heterochromatin at the centromeric region in Gorilla gorilla (GGO 11) and in Pongo pygmaeus (PPY 9), but this is compatible with a normal centromeric activity of these chromosomes.

Lejeune et al (1968) and Dutrillaux (1975) have observed that chromosome 2 in man occasionally shows a "lacune" (gap) at the region which corresponds to the inactivated centromeric region, and that this site may become the place from which selective endoreduplication occurs of the long arm of chromosome 2.

(B) How important was chromosomal change in speciation in the Hominidae?

The fact that phylogenetically related species show similarities in their karyotypes, and the fact that their karyotypes can be derived from each other by chromosome rearrangement opens the question as to whether such changes led to speciation or whether they have occurred after speciation. Mayr (1963) has defined a species as a population which is separated from others by discontinuity; the three main isolating mechanisms producing discontinuity being (a) geographical, (b) ecological and (c) reproductive. It is obvious that the ultimate mechanism which keeps species as separate taxa is reproductive isolation. The fact that human genes are only exchanged between human beings and not between human beings and chimpanzees keeps both species separate; otherwise, both species would blend into one. It is a matter of controversy, however, how important geographical isolation has been in speciation, and whether in fact, reproductive isolation can arise in the absence of geographical isolation. Mayr (1963) considers geographical isolation as the essential mechanism by which speciation has occurred, other isolating mechanisms having developed secondarily. These secondary mechanisms may be later capable of keeping the new species separate in the event that they may be brought together again in contact. These secondary mechanisms may act at the pre, intra or post-mating levels, but their main effect is to avoid cross hybridisation between different species, so that each of them may conserve its own genetic pool. Thus, the role of chromosome change in speciation is categorically denied by Mayr (1963), since chromosomal change has occurred, in his opinion, after speciation was produced by geographical isolation. This model of speciation has been named "allopatric", and it has been objected to on the grounds that some animal populations, which have been geographically/

geographically isolated for long periods, may belong to the same species, and may produce normal and fertile offspring if brought again into contact. This is the case of the polar bear (Thalarctos maritimus) and the brown bear (Ursus arctos), or the red deer (Cervus elaphus) and the wapiti or elk (Cervus canadiensis) (Short, 1976). Another objection to the allopatric model of speciation, this time in relation to human evolution, can be inferred from the findings of Leaky and Walker (1976). A hominid cranium with an endocraneal volume of 800-900 cc and with a great similarity to that of the Pekin Homo erectus was found at the same stratigraphic interval as Australopithecus boisei a more primitive hominid. This finding indicates that two contemporaneous hominid lineages coexisted at East Rudolf in East Africa. Thus, it might well be that geographical isolation was not the main cause of speciation in man, as it might not be of other organisms in which other mechanisms have been proposed. White (1968, 1973) has suggested that speciation may follow a "stasipatric" model; i.e. that species may have emerged whilst sharing a common habitat as a consequence of infertility barriers which gradually developed between populations which had become chromosomally different. Thus, in the stasipatric model, chromosome change is the main factor of speciation. One of the objections to this theory, however, is whether infertility barriers might restrict chromosomal change to those individuals in which it occurs, especially in complex organisms such as mammals. Our findings in Pongo pygmaeus in which one rearranged chromosome has been reported to be widespread in two subpopulations for at least 8,000 years are a clear indication that chromosomal change may not necessarily be restricted by infertility barriers in the heterozygous condition. However these findings do

not/

not prove that stasipatric speciation has occurred. Wilson et al (1974a, b, 1975) has observed that in mammals the rate of chromosomal evolution is higher than in other taxa, and have proposed that this might be explained by the way mammalian species and populations are socially organized, and in which a high amount of inbreeding and genetic drift may occur. In fact, both models of speciation, allopatric and stasipatric, may have contributed to the evolution of mammals. Arnason (1972) has compared the evolution of the Cetacea and Pinnipenidae with that of the Insectivora and Rodentia, and concluded that the former have probably evolved following an allopatric model of speciation, whilst the latter have evolved following a stasipatric model. As a general rule for mammalian species, Arnason (1972) commented that allopatric speciation must have occurred in those species showing high karyotypic stability, which correspond to those having:

(i) Low reproduction rate
- Late sexual maturity.
- Reduced litter size and few litters per year.

(ii) Good motility.

(iii) Environment without delimited niches.

On the other hand, stasipatric speciation applies well to species showing:

(i) High reproduction rate
- Early sexual maturity.
- Large litters and many litters per year.

(ii) Restricted motility.

(iii) Closely delimited ecological niches.

The Hominidae seems to correspond to the first group of mammals, and/

and this in turn must suggest that speciation has probably been allopatric rather than stasipatric. Our findings in two subpopulations of Pongo pygmaeus may be interpreted in favour of geographical isolation as necessary to precede fixed chromosome change. The fact that the two subpopulations differed in the frequency of one chromosome type (the Bornean and Sumatran chromosome 2) suggests that geographical isolation is needed so that genetic drift or selection may operate. On the other hand, the fact that a variant chromosome 9 is widespread in both subpopulations supplies good evidence that chromosome rearrangements do not lead to speciation "per se". These observations, however, do not prove that allopatric speciation is either taking place in present populations of Pongo pygmaeus, or has occurred in the Hominidae.

Ohno (1970) has commented on the nature of chromosome change in evolution and concluded that it is a rather conservative process which occurs within strict limits. In fact, we have observed that a large number of chromosomes are basically unmodified or have suffered only minor change during the divergence of the Hominidae, and that the original linkage groups of the subfamily Hominidae have also been conserved, as indicated by data on comparative gene mapping (Baltimore Conference, 1975). King and Wilson (1975) have demonstrated that structural genes of the chimpanzee and man are practically identical, and that the slow rate of change in structural loci is contrasted by a very rapid change in organic characters. Thus, King and Wilson (1975) have proposed that the main evolutionary mechanism which accounts for different rates of evolution in man and the higher primates, or between mammals and frogs (Wilson et al, 1974 a, b) does not rely on the divergence of structural loci due to mutation/

mutation, but rather of regulatory genes. Thus, if chromosome change were the main mechanism in producing speciation one of the ways it could have acted is by producing position effects, i.e. by affecting gene action due to location in the genome. Bodmer (1975) has suggested that a change in the order of genes might result in an arrangement which provides a selective advantage, and this order of genes could then become fixed in the population.

(C) The question of which species of great ape shows a greater similarity to man in its chromosome constitution, and which is the one phylogenetically closer to man as demonstrated by cytotaxonomy is a point of controversy. In the first place, some taxonomists consider phyletic affinity as equivalent to the degree of resemblance or similarity of characters which are equally weighted when species are compared. This is the approach of the phenetic school, or of numerical taxonomy (Sokal and Sneath, 1963). This approach, however, overlooks the fact that some characters compared might be positively correlated, or that characters which are present in two species may have resulted from convergence or parallelism rather than by the retention of true homologous characters (Simpson, 1974). In taxonomy, the word "homologous" refers to characters which have been retained from a common ancestor. Homologous characters could be "plesiomorph" if their origin can be traced to the primitive ancestor of the group, or "apomorph" if they have appeared in a later ancestral stock (see Martin, 1974). As an example in cytotaxonomy, the fact that all species of great ape and Hylobates lar and Hylobates moloch show an identical X chromosome is an indication that this is a plesiomorph character of the subfamily Hominidae, since this chromosome/

chromosome was probably present in the ancestral stock. The fact that brilliant fluorescence is present in four species of the Hominidae, but absent in one, as well as absent in the Hylobatidae and all other primates, suggests that this is an apomorph character which appeared more recently in time. Thus, the fact that two species may resemble each other very closely may in fact result from characters which were acquired later in evolution.

Martin (1974) has remarked that when a cluster of species is compared (A, B, C; Fig.7.2) it is insufficient to state that B and C are more closely related to one another and less closely related to A because the former two species have a larger number of characters in common. This could in fact result from sharing exclusive characters by B and C which were present in the common ancestor of B and C, but not in the common ancestor of A, B and C. If B and C were more closely related to each other than either were to A, we would expect them to share more homologous (plesiomorph) characters. However, this might not necessarily be the case, if some plesiomorph characters were lost or greatly modified through evolution. We may find a cluster of three living species, for example (Fig. 7.2) in which A and B share the greatest number of plesiomorph characters but B and C are the most closely related, having diverged from a more recent ancestral form. This comparison makes the distinction between "patristic" and "cladistic" relationship which results as a consequence of a different rate of divergence. In Fig.7.2 for example, the common retention of 50% of plesiomorph characters between A and B (patristic relationship) might obscure the fact that species C and B have diverged later from a common ancestor (cladistic relationship). This is due to the fact that species C has evolved very/

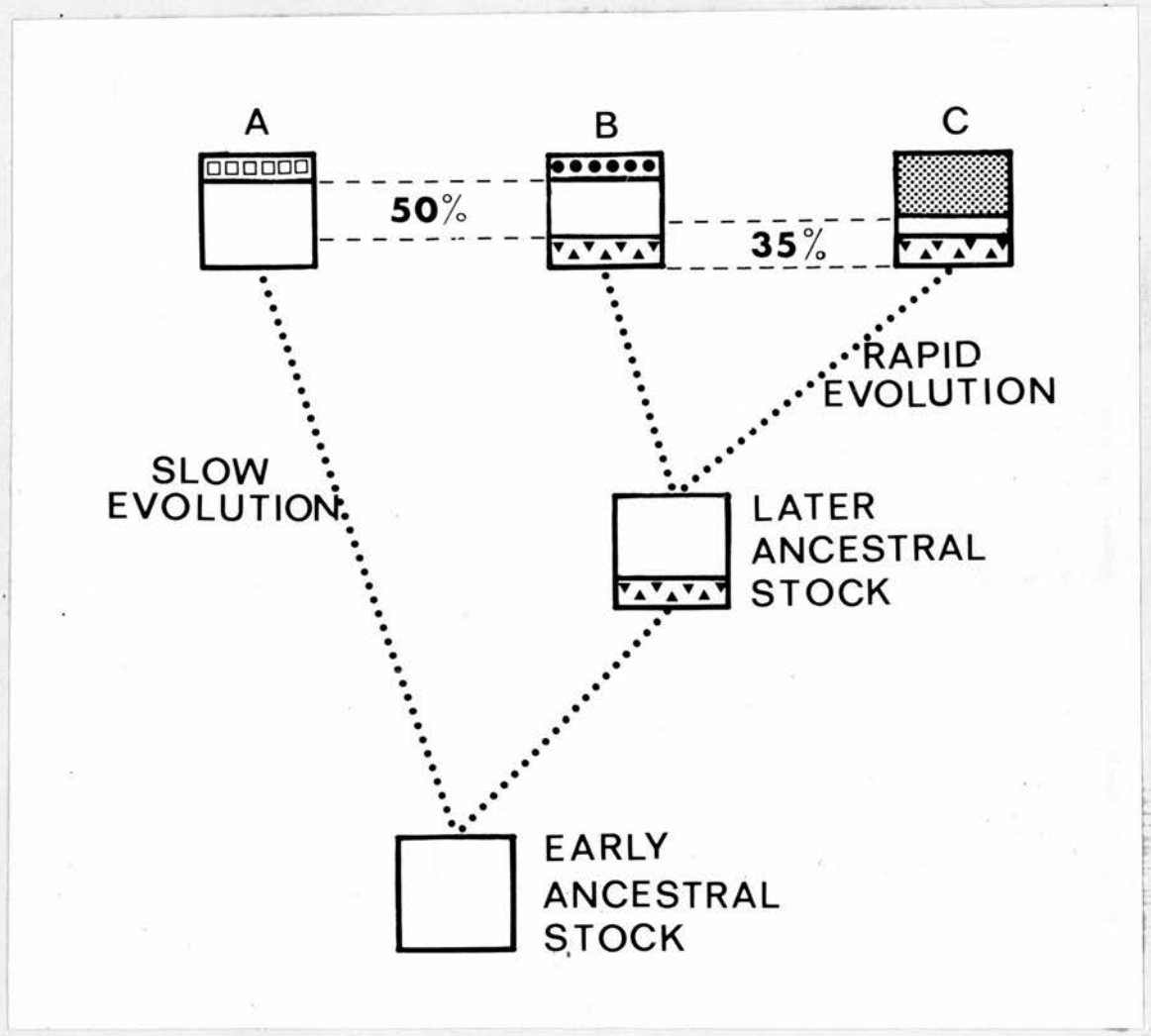



Fig.7.2. Cluster of three related species (A, B and C) sharing common characters (expressed in percentages). Plesiomorph characters are represented in white, apomorph characters in black triangles. From Martin, R. (1974).

very rapidly after its divergence from B, B and C only sharing 35% of common characters. Of this 35% only 10% correspond to plesiomorph characters, whereas 25% correspond to apomorph characters.

The following characteristics of the chromosome complement of the Hominidae could be considered as those of the common ancestor of the group (Fig.7.3):

1. Diploid chromosome number = 48.
2. No brilliant fluorescence.
3. No interstitial C-band regions.
4. No secondary constrictions in metacentric chromosomes
5. No terminal C-band regions.
6. No satellite DNA sequences (only single or few copies per genome of each sequence).
7. X chromosome identical to that of any modern species of the Hominidae.
8. Small Y chromosome.
9. All centromere regions positively C-banded.

In this comparison we will consider that the retention of the ancestral chromosomes represents the retention of plesiomorph characters, whereas chromosomal change represents characters which were acquired later in the Hominidae. It is then important not only to know what change has occurred within the group, but to distinguish the number of possible chromosome rearrangements that have occurred between species. For this comparison, we will only consider chromosomal changes that have involved euchromatic regions of the Hominidae.

Man-Pan troglodytes: 2 pericentric inversions + one telomeric fusion

Man-Pan paniscus: 2 pericentric inversions + one telomeric fusion

Man-*Gorilla gorilla*: 5 pericentric inversions + one telomeric fusion.

Man-*Pongo pygmaeus*: 3 pericentric inversions + one paracentric inversion + one telomeric fusion.

*Pan troglodytes*-*Pan paniscus*: None.

*Pan troglodytes*-*Gorilla gorilla*: 2 pericentric inversions.

*Pan paniscus*-*Gorilla gorilla*: 2 pericentric inversions.

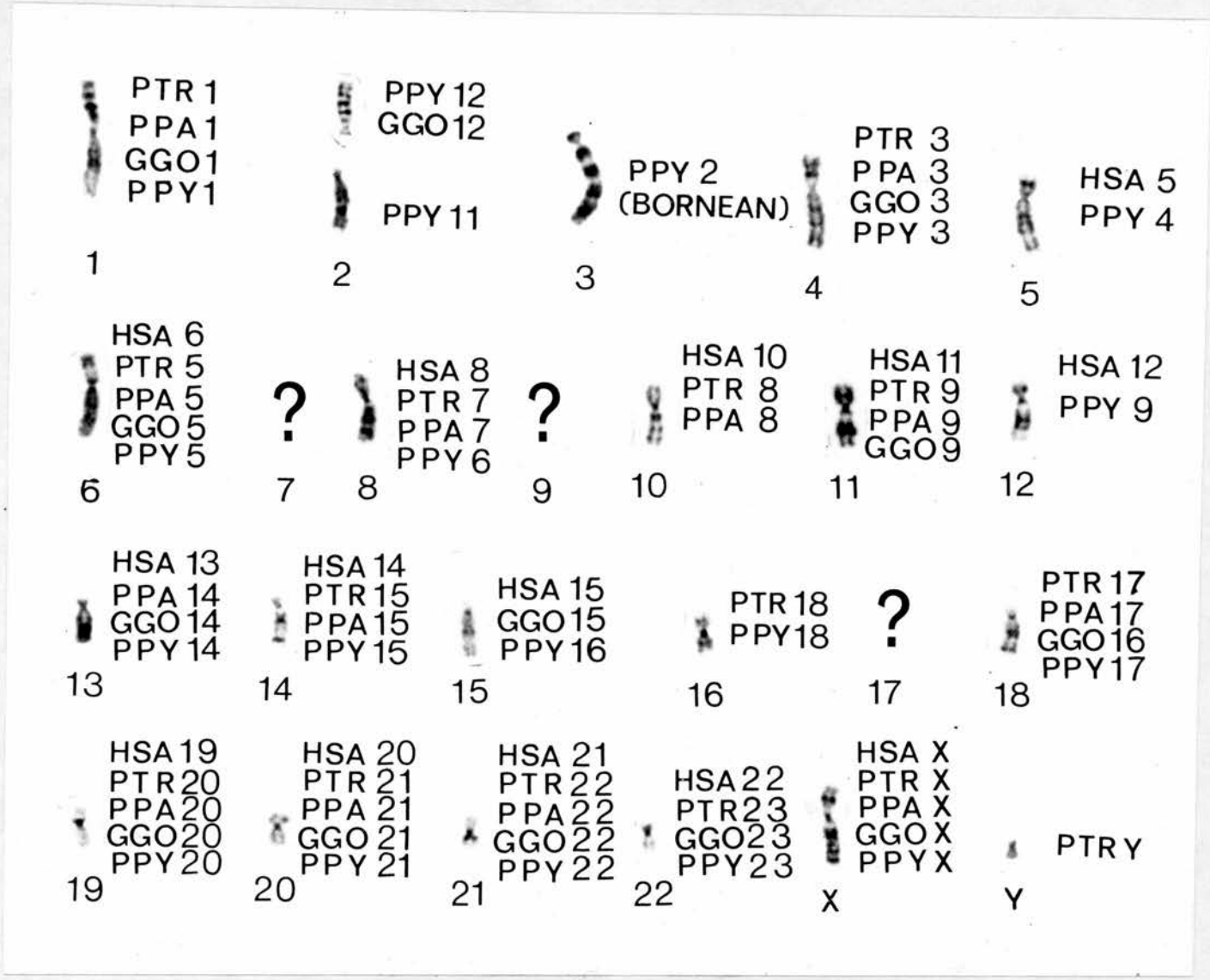
*Pan troglodytes*-*Pongo pygmaeus*: 4 pericentric inversions + one paracentric inversion.

*Pan paniscus*-*Pongo pygmaeus* 4 pericentric inversions + one paracentric inversion.

*Gorilla gorilla*-*Pongo pygmaeus*: 6 pericentric inversions + one paracentric inversion.

The highest number of chromosome rearrangements (7) are those between *Gorilla* and *Pongo*, and the second highest is that between *Gorilla* and man (6). Very surprisingly, the latter have a higher number than Man and *Pongo* (5), although there is clear evidence that *Pongo* has split from the Hominidae before the splitting of the African apes and man as estimated by immunological distances (Goodman, 1974) and experiments of *in vitro* DNA hybridisation (Beneviste and Todaro, 1976). The fact that man and *Pongo* show less chromosome change, and have retained plesiomorph characters such as the absence of telomeric C-band regions (patristic relationship) apparently obscures the fact that man and *Gorilla* have diverged later from a common ancestor (cladistic relationship). This is obviously due to the fact that the rate with which chromosomal change has occurred has not been the same between all species, but the chromosomes of *Gorilla* diverged much more rapidly from the chromosomes of man than did the chromosomes of *Pongo*, although this species had separated earlier in time. A comparison between *Pan troglodytes* and *Pongo* shows less number of chromosome/

Fig.7.3. Ancestral G-band chromosome complement of the Hominidae arranged according to anthropomorphic criteria. Numbers below the chromosomes denote human equivalents to which the illustrated chromosomes are homologues. Symbols at the right side denote the individual chromosome of each species whose G-band pattern is similar to that of the proposed ancestor.


PTR 1
PPA 1
GGO 1
PPY 1
1
PPY 12
GGO 12
PPY 11
2
PPY 2
(BORNEAN)
3
PTR 3
PPA 3
GGO 3
PPY 3
4
HSA 5
PPY 4
5
HSA 6
PTR 5
PPA 5
GGO 5
PPY 5
6
?
7
HSA 8
PTR 7
PPA 7
PPY 6
8
?
9
HSA 10
PTR 8
PPA 8
10
HSA 11
PTR 9
PPA 9
GGO 9
11
HSA 12
PPY 9
12
HSA 13
PPA 14
GGO 14
PPY 14
13
HSA 14
PTR 15
PPA 15
PPY 15
14
HSA 15
GGO 15
PPY 16
15
PTR 18
PPY 18
16
?
17
PTR 17
PPA 17
GGO 16
PPY 17
18
HSA 19
PTR 20
PPA 20
GGO 20
PPY 20
19
HSA 20
PTR 21
PPA 21
GGO 21
PPY 21
20
HSA 21
PTR 22
PPA 22
GGO 22
PPY 22
21
HSA 22
PTR 23
GGO 23
PPY 23
22
HSA X
PTR X
PPA X
GGO X
PPY X
X
PTR Y
Y

chromosome rearrangements (5) than between Gorilla and Pongo (7), whereas only 2 rearrangements have occurred between Pan troglodytes and Gorilla. There is good evidence that these two species are more closely related between themselves than any of them is to Pongo (Goodman, 1974, Beneviste and Todaro, 1976). Thus, the difference in the number of chromosome rearrangements suggests that the rate of chromosome change between Pan troglodytes and Pongo has been slower than between Gorilla and Pongo. The most logical explanation is that the chromosomes of Pan troglodytes have evolved less rapidly than those of Gorilla, thus retaining more plesiomorphic characters (patristic relationship). It is not surprising, then, that only 3 chromosome rearrangements have occurred between Man and Pan troglodytes, whereas 6 have occurred between man and Gorilla. This patristic relationship between man and Pan troglodytes might in fact obscure the fact that man and Gorilla might be the most closely related species, which will be shown by the retention of apomorph and not plesiomorph characters. Thus, it is important to establish which will be the apomorph characters of any pair of species, should they have emerged from a common ancestor.

Man-Pan troglodytes: None.

Man-Gorilla gorilla:

1. Y chromosome with brilliant fluorescence.
2. One homologous pair with brilliant fluorescence (HSA 4 and GGO 3).
3. Secondary constrictions at two homologous chromosome pairs (HSA 9 = GGO 18; HSA 16 = GGO 17).
4. 5-methylcytosine rich regions in four homologous chromosomes (HSA 9 = GGO 18; HSA 15 = GGO 15; HSA 16 = GGO 17; HSA Y = GGO Y).

Pan/

Pan troglodytes-Gorilla gorilla: 1. Terminal Q- C-bands.

2. Two pericentric inversions; one HSA 5, PPY 4 → PTR 4, PPA 4 and GGO 4; the other HSA 12, PPY 9 → PTR 10, PPA 10 and GGO 10.

Thus it appears that either man and Gorilla or Pan troglodytes and Gorilla have diverged from a recent common ancestor in the Hominidae, but no recent common ancestor seems to have existed between man and Pan troglodytes. The indication that man and Gorilla might have a common ancestor comes from the fact that both species show brilliant fluorescence at the same regions of homologous chromosomes (e.g. the subcentromeric region of HSA 4 and GGO 3, and the distal long arm of the Y chromosome), and that the appearance of secondary constrictions has taken place exclusively in these two species and in particular in the two homologous chromosomes HSA 9 and GGO 18; HSA 16 and GGO 17. Another indication is the fact that in these two species there are detectable amounts of 5-methylcytosine rich DNA sequences which are distributed at the same chromosome sites. In man, methylated DNA sequences have been detected by immunofluorescence techniques at the C-band regions of chromosome 1, 9, 15, 16 and the Y chromosome (Miller et al, 1974b). In Gorilla gorilla these sequences have been detected at the C-band region of chromosomes 12, 13, 14, 15, 17, 18 and the Y chromosome (Schnedl et al, 1975), whereas in Pan troglodytes none of the chromosomes showed detectable amounts of 5-methylcytosine rich regions. Similar studies in Pongo pygmaeus are needed to clarify this point completely. This is why the absence of detectable amounts of these sequences in Pan troglodytes cannot be taken as proof in favour of a common ancestor man-Gorilla gorilla. However, what suggests that man and Gorilla gorilla might have had a common ancestor is the distribution/

distribution of the 5-methylcytosine rich regions in their karyotypes. The fact that these regions are located at many homologous chromosomes of man and Gorilla gorilla (HSA 9 and GGO 18; HSA 15 and GGO 15; HSA 16 and GGO 17; HSA Y and GGO Y) indicates that the amplification of these sequences could have occurred in a common ancestor of both species. Otherwise we would have to postulate that the similar distribution of these DNA sequences in these two species has resulted from a random event.

Thus, the chromosomes of man and Gorilla gorilla may differ in the number of chromosome rearrangements which have occurred between them, but in spite of these changes the overall phyletic affinity between the karyotype of Gorilla gorilla and man is greater than between Pan troglodytes and man contrary to the generally held view that the chimpanzee is man's closest living relative.

## (E) Summary.

(1) The chromosomes of man and the great apes have been compared and chromosome homology has been inferred from different degrees of similarity.

(2) Chromosome rearrangements have been postulated to have occurred within the Hominidae; and the pericentric inversion has been found to be the most common type of rearrangement within the group.

(3) The role of chromosome rearrangement in speciation in the Hominidae has been discussed.

(4) The degree of phyletic affinity between man and the great apes, as indicated by cytotaxonomy, has been examined. This study has indicated that a larger number of chromosome rearrangements has occurred between man and Gorilla gorilla than between man and Pan troglodytes. The gorilla, however, appears to be the most closely related primate species to man, both species probably having emerged recently from a common ancestor.

## SECTION II

## THE SPERMATOZOA OF THE GREAT APES AND MAN

## CHAPTER 8: GENETIC AND MORPHOLOGICAL STUDIES OF SPERMATOZOA.

Human and animal spermatozoa were initially described by Leeuwenhoek in 1678 as small animals with a head and a tail,and in the following century the experiments conducted by Lazaro Spallanzani demonstrated that spermatozoa were needed for fertilization. By the mid XIXth Century spermatozoa were recognized as highly specialized cells differentiated from the testicular epithelium (Kølliker, 1841; Schweigger-Seidel, 1865; la Valette St. George, 1885), and in 1876 Otto Hertwig demonstrated that fertilization consisted of the penetration of the egg by the spermatozoon followed by the fusion of their nuclei. The development of the science of genetics in the early XXth Century, the chromosomal theory and the understanding of the mechanisms of sex determination supplied conclusive evidence that the spermatozoa in the ejaculate comprised a genetically heterogeneous cell population. Furthermore, in species in which the male was the heterogametic sex, it was evident that spermatozoa comprised two chromosomally different classes, the X-bearing and the Y-bearing.

### General findings in animal spermatozoa.

Extensive studies on the morphology of spermatozoa of many animal species were made by Retzius. Included in his studies were the first reports on the spermatozoa of *Pan troglodytes* (1911), *Gorilla gorilla* (1913) and *Pongo pygmaeus* (1910). More recently, studies of spermatozoa of many different species have been made (Fawcett, 1970; Bedford, 1974; Baccetti and Afzelius, 1976; Austin, 1976)/

1976), and all agree that the spermatozoon is a highly specialized cell with distinct morphological, biochemical and behavioural attributes which vary substantially between species. In general, however, it has been observed that there is a better relationship between morphology and function than between morphology and phylogenetic affinity(Baccetti and Afzelius, 1976), although it is possible to find that some attributes of the spermatozoon have been substantially modified during the course of evolution. The male gamete has successfully adapted to different situations which have required the modification of its primary structure. In animals the spermatozoon is an aquatic cell with a motility of its own, most frequently possessing a flagellum. In terrestrial animals spermatozoa are no longer released into the external environment but they are restricted to the internal fluids and secretions, a factor that has increased their complexity. Thus, two basically different types of spermatozoon are found in the animal kingdom, one which is recognized as "primitive" consisting of a small cell with a round nucleus with radial symmetry surmounted by an acrosome, a short midpiece with a few mitochondria and a tail. The tail shows the simplest 9+2 type of flagellum, and the most highly specialized region of this spermatozoon is its acrosomic cap which shows substantial variation between species (Fawcett, 1970). A second type of spermatozoon is that which has developed in animals with internal fertilization. The head has become more elongated and the midpiece has increased in size becoming reorganized around the flagellum, a change which is related to a more efficient energy generating mechanism. The spermatozoa of mammals, especially those of eutherian mammals are an example of a modified type of cell in which/

which a high degree of specialization has been achieved. One important function of the eutherian spermatozoon is its capacity to penetrate the tough zoma pellucida, a fact that explains the tapered configuration of the head. Fawcett (1965) has defined the basic morphological components of the eutherian spermatozoon as (i) the head with the nucleus and acrosome, (ii) the neck with the basal plate, the connecting piece and the persisting centriole, (iii) the mid piece defined by the mitocondrial sheath, and (iv) the main or the principal piece of the tail.

Genetic aspects of spermatozoa.

The fact that there are distinct differences in spermatozoal morphology between species strongly suggests that there is genetic control of spermatozoal attributes, and extensive reviews of evidence in support of this concept have been carried out by Beatty (1970, 1975). Genetic control of morphology is especially evident between strains within a species, such as the rabbit or the mouse (Braden, 1958; Napier, 1961; Wooley and Beatty, 1967). It must be pointed out, however, that the heritability values for spermatozoal dimensions vary considerably; that for mid piece area was almost zero whereas for midpiece length it was 0.97. This high value was confirmed in a classical genetic selection experiment in which male mice were selected for mid piece length, and two substrains were created each of which had a characteristic mid piece length (Wooley and Beatty, 1967; Wooley, 1970). The important conclusions of these experiments and of previous observations on the heritability of many spermatozoal dimensions are (i) that spermatozoal attributes are extremely constant in spite of many environmental/

environmental and biological sources of variation (Beatty and Sharma, 1960), and (ii) that characters associated with high values of heritability are totally unrelated to a high reproductive efficiency, as previously demonstrated by Falconer (1964).

The determination of spermatozoal phenotype.

The attributes of the animal spermatozoon are genetically determined by the diploid genotype of the organism, but are independent of its own haploid genetic content, since post segregational gene action does not occur (Beatty, 1975). This characteristic of animal spermatozoa contrasts sharply with the plant kingdom where haploid gene expression is observed. This particular aspect of the animal spermatozoon can be demonstrated in the following situations: (i) recessive genetic mutants exist in mice and cattle which are responsible in the homozygous condition for the production of abnormal spermatozoa (Donald and Hancock, 1953; Hollander et al, 1960; Bryan, 1968). However, normal spermatozoa are produced by heterozygous male animals, although half their spermatozoa must carry the abnormal mutant gene; (ii) In Drosophila melanogaster normal offspring result when nullisomic males are mated to females disomic for the same chromosome (Lindsley and Grell, 1969), emphasising that the formation and fertilizing capacity of spermatozoa were not impaired by gross genetic imbalance; (iii) in $F_1$ interspecific male hybrids between Mus musculus: 2n = 40, NF = 40, and Mus poschiavinus: 2n = 26, NF = 40, a large number of secondary spermatocytes are chromosomally unbalanced as a consequence of abnormal meiotic segregation. Microdensitometric analysis of the spermatozoa of such hybrids shows a significant increase in the variance/

variance of DNA measurements compared with that observed in animals of the two parental species (Doring et al, 1972). However, a very large proportion of these presumably aneuploid spermatozoa are morphologically normal. Moreover, the distribution of counts of chromosome arms in preimplantation embryos of a backcross between an $F_1$ male hybrid and a female Mus musculus were concordant with the distribution found in secondary spermatocytes of the same hybrid male animal. This suggests that chromosome imbalance does not affect the production and fertilizing capacity of the spermatozoon (Ford, 1972); (iv) when mice from strains differing in spermatozoal morphology are crossed, the variance of these attributes in $F_1$ males is no greater than that found within animals of each strain (Sharma, 1960 ; Pant, 1972), although heterozygous animals must segregate many different kinds of spermatozoal genotypes; (v) it has been shown that in the mouse RNA synthesis does not occur after meiotic segregation, and that protein synthesis during spermiogenesis utilizes DNA previously transcribed at pre-reductional stages (Monesi, 1962, 1971).

Evidence of post segregational gene expression in animal spermatozoa.

Although post segregational gene action does not normally take place, there are few rare exceptions in which it does occur. One of these is the T (tailess) locus in mice and the SD (segregation distorter) locus in Drosophila melanogaster. In mice, Braden (1958) found that Tt heterozygous male animals produced a significant excess of tt offspring. This could not be explained by a higher mortality of Tt embryos. The situation reverted to a normal Mendelian ratio when delayed matings occurred, a finding which ruled out the possibility/

possibility that more t bearing spermatozoa were produced. The logical explanation was that t bearing spermatozoa were more fertile than T spermatozoa in the first instance. Thus, this seems to be one of those cases where spermatozoal fertility is correlated with the haploid genotype. The SD locus in Drosophila (Sandler et al, 1959) is another instance in which the wild type allele (SD+) is transmitted to the offspring at a significantly lower ratio than expected. Peacock et al (1972) have studied this problem under the electrom microscope and found that spermatids carrying the SD+ allele degenerate as a consequence of a lethally induced effect by SD on SD+. However, it was found that although the lesion was haploid genome specific, it depended on the diploid genotype and on pairing of homologous chromosomes, all factors that point to the fact that gene action must start at the primary spermatozyte stage, but continue after meiotic segregation.

Possible cases of post-segregational gene action in human spermatozoa.

There is no conclusive evidence that post-segregational gene expression occurs in man, but some findings suggest that this might be the case. One is the claim that AB blood group males produced two kinds of antigenically distinguishable spermatozoa, those with the A and those with the B antigen (Gullbring, 1957; Shahani and Southam, 1962). Somewhat similar situations were reported for men heterozygous for histocompatibility antigens (Fellous and Dausset, 1970), although these results have not been confirmed by the majority of investigators, and can be criticised on the grounds that antigens detected in spermatozoa might be derived from the seminal plasma rather than from the spermatozoon. Edwards et al (1964) found A and

B/

B substances in semen of secretors only, and inferred that these antigens were secondarily adherent to the spermatozoa. Other investigators, however, (see Ackerman, 1969) claimed that AB substances may be found in spermatozoa of both secretors and non secretors. Moreover, Shahani and Southam (1962) and Popianov and Vulchanov (1962) reported that two antigenically different spermatozoal populations (A and B) could be found in both AB secretors and non secretors. This conflicting evidence is furthermore complicated by inconclusive results in other mammalian species such as the rabbit (Beatty and Cohen, 1957); the generally held view is that proof of post segregational gene action based on antigenic properties of human spermatozoa is still insufficient (Beatty, 1970). Enzymatic determinations of glucose 6-phosphate dehydrogenase, an X-linked gene, has been performed in human spermatozoa, and it was initially claimed that there was cytochemical evidence for differential enzyme activity (Edwards and Valentine, 1963). More recently, Sarkar et al (1977) have observed marked differences in the activity of this enzyme using fluorimetric techniques to detect the amount of NADPH formed inside spermatozoa, although it was technically impossible to identify the Y chromosome (by demonstration of an F body) in cells with low enzymatic activity. Cohen (1971) has postulated that haploid gene expression results as a consequence of genetically imbalanced spermatozoa resulting from defective cross-over mechanisms, but this hypothesis has been subject to strong criticism (see Wallace, 1974).

Properties of X-bearing and Y-bearing human spermatozoa.

There/

There is no evidence that X-bearing and Y-bearing human spermatozoa are morphologically different, contrary to the initial claims of Shettles (1960). There is also no consistent evidence in other mammalian species that two morphologically different classes of spermatozoa can be distinguished in the ejaculate (Beatty, 1960, 1970). There have been claims that non-equilibrium sedimentation of spermatozoa can result in a significant departure from the expected 1:1 sex ratio in the rabbit and the bull although results were inconsistent and departures from the expected sex ratio were not always in the same direction (Beatty, 1970). In man, it has been reported that Y-bearing spermatozoa are more abundant in the front zone migration of cervical mucus of women (Rhode et al, 1973), and that a rich Y bearing fraction could be isolated in albumin density gradients as a consequence of the higher motility of Y-bearing spermatozoa (Ericsson et al, 1973). This latter observation was not confirmed (see Ross et al, 1975 and Evans et al, 1975), although more recently it has been claimed that successful separation can be achieved in a Ficoll-sodium metriozate density gradient (Shastry et al, 1977). Rohde et al (1975) reported a successful centrifugation separation of human spermatozoa in discontinuous sucrose gradients; the detection of the Y-bearing spermatozoa being higher in the lighter fractions as indicated by a higher percentage of cells with a visible fluorescent body. Other techniques such as electrophoresis which attempted to isolate these two kinds of spermatozoa in man and animals have given inconsistent results (Beatty, 1970).

Substantial progress, however, has been achieved in identifying Y-bearing spermatozoa in man due to the fact that the terminal/

terminal region of the Y chromosome stains brilliantly with quinacrine fluorescence (Zech, 1969). This peculiar property of the human Y chromosome, and the fact that other brilliant fluorescent regions of the human chromosome complement, if present, are usually considerably smaller in size, has permitted the identification of the Y chromosome in spermatozoa (Barlow and Vosa, 1970) as a brilliant ("F") fluorescent body. Sumner et al (1971b) demonstrated that spermatozoa with one visible F body had a lower DNA content than those with no visible F body, and that the difference between the mean DNA content of the two populations coincided with the difference of DNA content between the medium sized human X chromosome and the small sized Y chromosome. The difference observed was not, however, sufficient to produce two separate mean values of DNA content when spermatozoa were measured using a flow system analyser after staining with a Feulgen fluorescent stain (acriflavin) (Sarkar et al, 1974). Thus, the identification of Y-bearing spermatozoa in man depends entirely on the presence of a visible F body following fixation and staining, although it has also been claimed that even this recognition may be biased by technical and observational factors which may produce unreliable estimates (Beatty, 1977).

The biology of human and primate spermatozoa.

From what has just been said, it can be appreciated that spermatozoal morphology is determined by the diploid genotype of the primary spermatocyte. This has been demonstrated by Burgoyne (1975) who found that in the chimaeric testes of mice two different kinds of spermatozoa were produced, each of which corresponded with that/

that produced by the strain of mouse used to make the chimaeric animal. This experiment proved that the germinal tissue rather than the somatic tissue is the site at which the spermatozoal attributes are determined. In eutherian mammals, the maturation of spermatids into spermatozoa involves complex structural and biochemical mechanisms. Gledhill (1970) has reported that in the bull the lysine containing histone is replaced by an arginine rich protein, as well as incorporation of cystein (Loir, 1970). Morphologically, the cells suffer a progressive condensation of their nuclei accompanied by moulding to a shape characteristic of the species. However, the spermatozoon which is released from the testes is far from being a mature product, but undergoes substantial structural changes during its passage through the epididymis (Bedford et al, 1972). In the first place, Bedford et al (1973 b) have reported that motility of human spermatozoa increases considerably between the caput and the cauda epididymis. The capacity of human, Rhesus monkey and baboon spermatozoa to bind ferric oxide colloidal particles at pH = 1.8 changes from practically nil at the caput to considerable activity at the cauda epididymis (Bedford et al, 1972; Bedford, 1974). However, the sites of colloidal binding were restricted to the post acrosomal region in the Rhesus monkey and the baboon whereas in man the whole acrosomic and post acrosomic region showed a marked binding capacity. It was observed that rabbit and rat spermatozoa would swell if treated with a 1% solution of dodecylsulfate (DDS) containing dithiothreitol (DDT) (Cleland's Reagent), since disulphide links are specifically cleaved (Calvin and Bedford, 1971). The degree of swelling observed in the rabbit and rat varied depending on the site of the epididymis where the sample/

sample of spermatozoa were obtained. Those from the caput epididymidis exhibited a greater degree of swelling than those from the cauda, a finding which suggested that disulphide links were established during passage through the epididymis. When spermatozoa from the vas deferens of the Rhesus monkey was exposed to Cleland's Reagent for 15 minutes, a very uniform degree of swelling was observed, but a very heterogeneous degree of swelling was observed under similar experimental conditions with ejaculated human spermatozoa (Bedford et al, 1972, 1973a). This result suggested that ejaculated human spermatozoa are made up of cells at different stages of maturation; the possible factors involved include either the mixing of mature and immature spermatozoa in the epididymis probably resulting from the small testis size in man, and poor epididymal reserves, in marked contrast to the Rhesus monkey (Short, 1977), or the fact that spermatogenesis in man might be affected by some environmental factor, e.g. clothing induced hyperthermia (Bedford et al, 1973a, Bedford, 1974).

Ultrastructure of human and primate spermatozoa.

A comparison of the spermatozoa of man and other primates including two species of the superfamily Hominoidea, Pan troglodytes and Hylobates lar showed that under the electrom microscope, human spermatozoa exhibited a remarkable degree of morphological pleomorphism which was absent from other primate species so far studied (Bedford, 1974). This finding coincided with previous observations of human spermatozoa under the light microscope (McLeod, 1970). Detailed studies of the human spermatozoal head showed that the shape of the nucleus was extremely variable with large vacuolated regions./

regions. These regions corresponded to sites of irregular packaging of chromatin, and these regions became more prominent when human spermatozoa were treated with the Cleland's Reagent (Bedford et al, 1973a).

A comparison between the spermatozoa of man and other primate species showed differences in morphology and in some other attributes. Spermatozoa of Prosimian species (genera Lemur, Galago and Nycticebus) showed intense agglutination and were unable to bind ferric colloidal particles, whereas those of the Anthropoidea did not show intense agglutination but were capable of binding ferric colloidal particles. The spermatozoa of man and Pan troglodytes showed a uniform pattern of colloidal binding all over the spermatozoal head, whereas in the Cercopithecoidea and Cebidae the site of colloidal binding was restricted to the post acrosomal region of the head (Bedford, 1974).

Studies with the Scanning electron microscope.

Martin et al (1975) made comparative studies of the spermatozoa of man and other primate species, including all species of great ape. This study again pointed out the morphological pleomorphism present in human spermatozoa, and showed a similar picture in Gorilla gorilla. Moreover, a comparison between species showed that the spermatozoa of man and Gorilla gorilla were extremely similar, making it difficult to distinguish between them. Pan troglodytes and Pan paniscus showed practically identical spermatozoa, and both species produced regularly shaped and sized spermatozoa which were clearly distinct from those of man, Gorilla gorilla or Pongo pygmaeus. In this latter species, the production of spermatozoa was also extremely uniform/

uniform, but morphologically distinct from the other species.

Comparative studies of spermatozoa of man and the great apes with the light microscope and with an integrating interferometer.

The first descriptions of the morphology of the spermatozoa of Pan troglodytes, Gorilla gorilla and Pongo pygmaeus were reported by Retzius in 1911, 1913 and 1910 respectively. Detailed spermiograms of the great apes as usually made for man with the Papanicolaou technique have recently been reported (Seuanez et al, 1977a), as well as studies of the distribution of brilliant fluorescent bodies in the spermatozoa of the great apes and man (Seuanez et al, 1976d). Estimations of the dry mass of spermatozoa of man and the great apes are included in the report of Seuanez et al, 1977a, and all these results will be presented in Chapters 9 and 10 of this Thesis.

# CHAPTER 9: VISUALIZATION OF BRILLIANT FLUORESCENT BODIES IN THE SPERMATOZOA OF THE GREAT APES AND MAN.

## (A) Introduction.

Human semen has long been known to differ from that of other mammalian species in the high proportion of morphologically abnormal spermatozoa that it contains (Bedford, 1974). However, there are very few reports in the literature in which the spermatozoa of man and his closest living relatives, the great apes, are compared. Early this century, Retzius (1910, 1911, 1913) made the first studies of the spermatozoa of the great apes (with the exception of *Pan paniscus*), and he was the first to notice similarities between the spermatozoa of man and the gorilla. In his opinion, these resembled each other more closely than either did the sperm of the chimpanzee or orangutan. These studies are summarized in a review of primate reproductive biology by Stark (1956). Martin *et al* (1975) made studies at the scanning electrom microscope level on the spermatozoa of man and a number of non-human primate species, among which were the four species of great apes. This study confirmed the marked morphological similarities between the spermatozoa of man and the gorilla. In this chapter we present the results of studying the spermatozoa of man and the great apes at the light microscope level using Nomarski interference, with open field using Papanicolaou stained preparations, and with the fluorescence microscope using quinacrine stained preparations. All individuals from which semen samples were obtained were of known fertility except one male orangutan (S.I.16, see Chapter 4) which had never been mated.

(B)/

(B) Morphology of spermatozoa of man and the great apes.

A comparative study of the spermatozoa of the Hominidae shows that man and Gorilla gorilla exhibit a remarkable morphological pleomorphism, unlike Pan troglodytes, Pan paniscus and Pongo pygmaeus where spermatozoa are regular in size and shape. In Pan troglodytes the head of the spermatozoon is rectangular and elongated with its longitudinal axis approximately twice the length of the transverse axis. With Nomarski interference (Fig.9.1) the morphology of the head is clearly seen to be different from that of man, Gorilla gorilla and Pongo pygmaeus, but identical to Pan paniscus, the pygmy chimpanzee (Fig.9.2). In Papanicolaou stained preparations the head of the spermatozoon from the two species of chimpanzee are intensely stained and regular in shape. The presence of vacuoles is the most common abnormality found, but the incidence of such an anomaly was very low (Table 9.1). In Pongo pygmaeus, spermatozoa showed great uniformity in size and appearance. The anterior portion of the spermatozoon appeared flat and laterally expanded; it corresponded to the region of the acrosome. A clear post acrosomic margin was evident, and below it, the rest of the nucleus appeared slightly constricted (Fig.9.3 and Fig.9.4). In the two individuals examined, virtually all the spermatozoa examined were of the "normal" cellular type.

In Gorilla gorilla, as in man, a remarkable morphological pleomorphism exists, and we have therefore defined "morphologically normal" spermatozoa as the most common (modal) cell type observed in the ejaculate. In Gorilla gorilla, the "normal" modal cell type is a regularly shaped oval cell, practically identical to the modal (normal)/

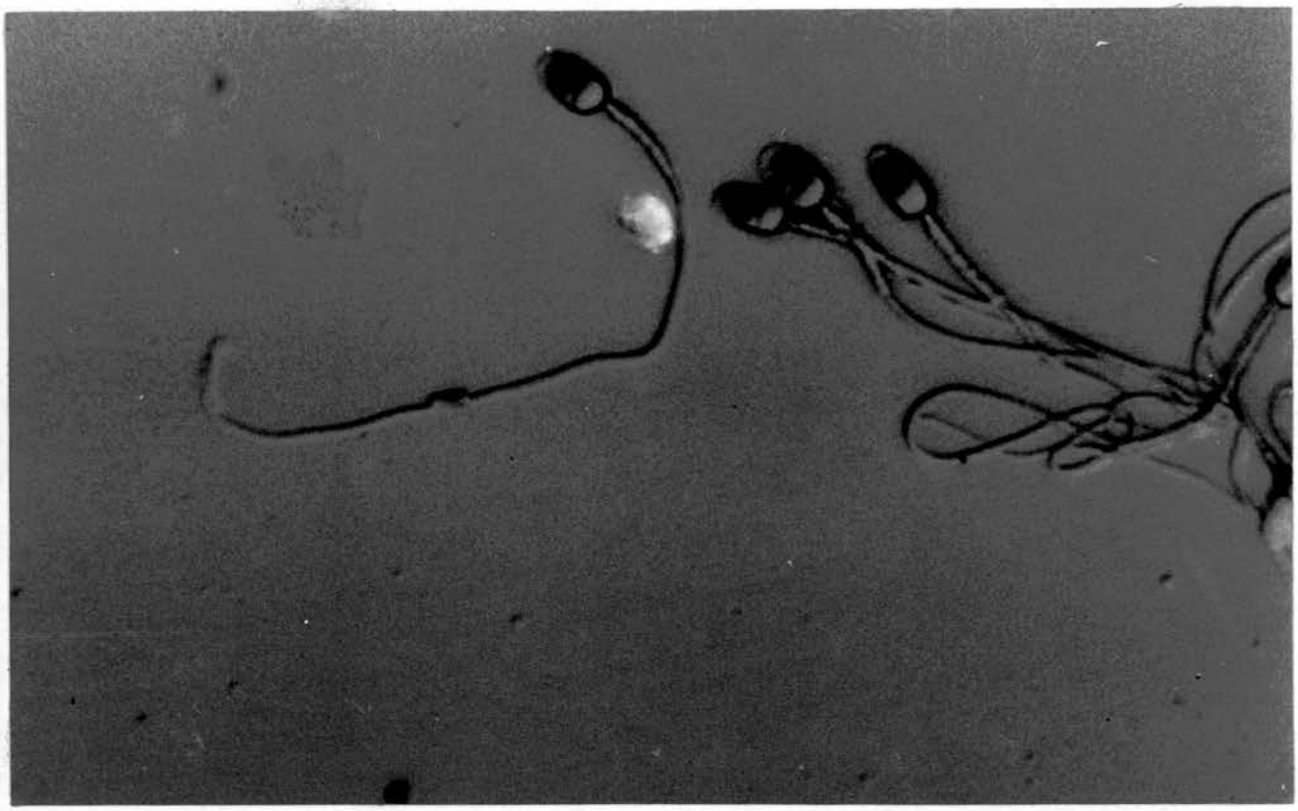


Fig.9.1. Spermatozoa of Pan troglodytes viewed with Nomarski interference. Scale bar 10mμ.

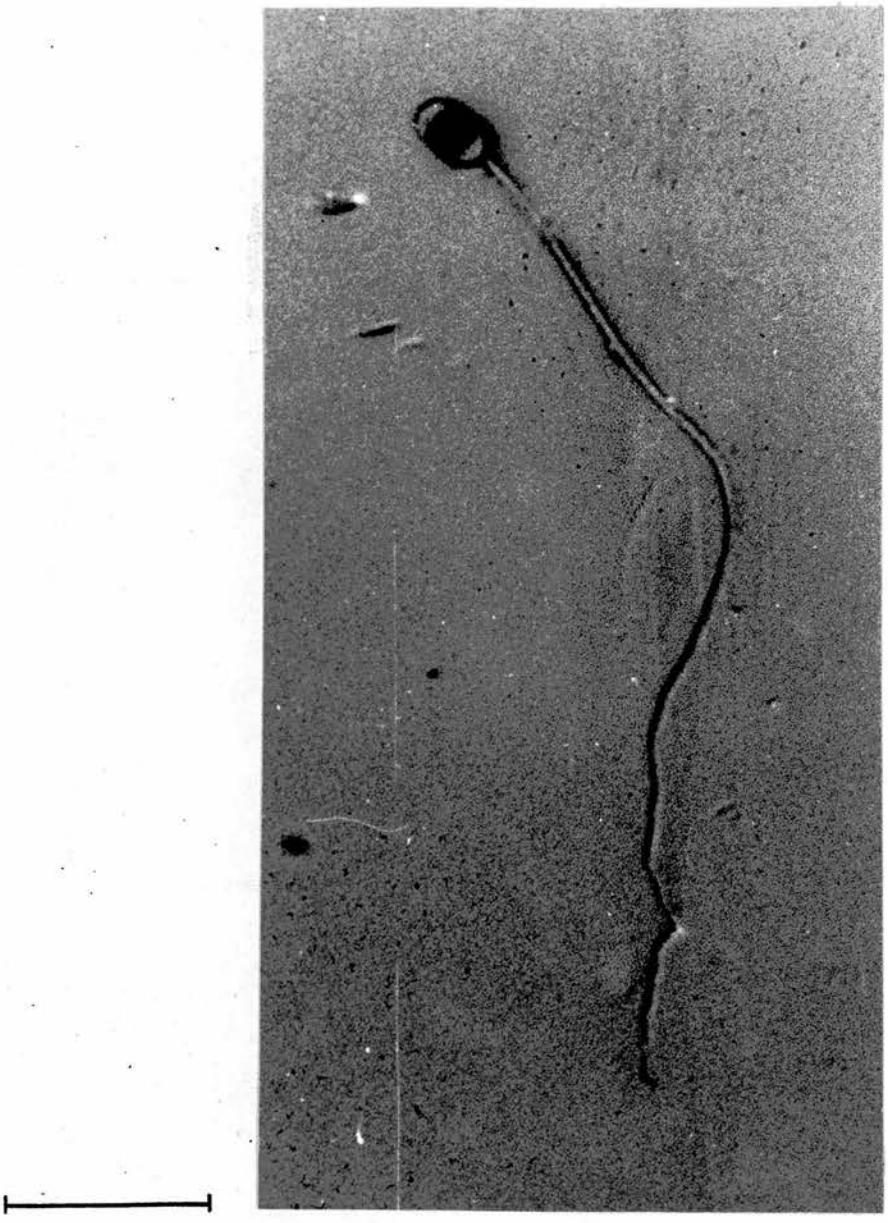


Fig.9.2. Spermatozoa of Pan paniscus viewed with Nomarski interference. Scale bar 10mμ.

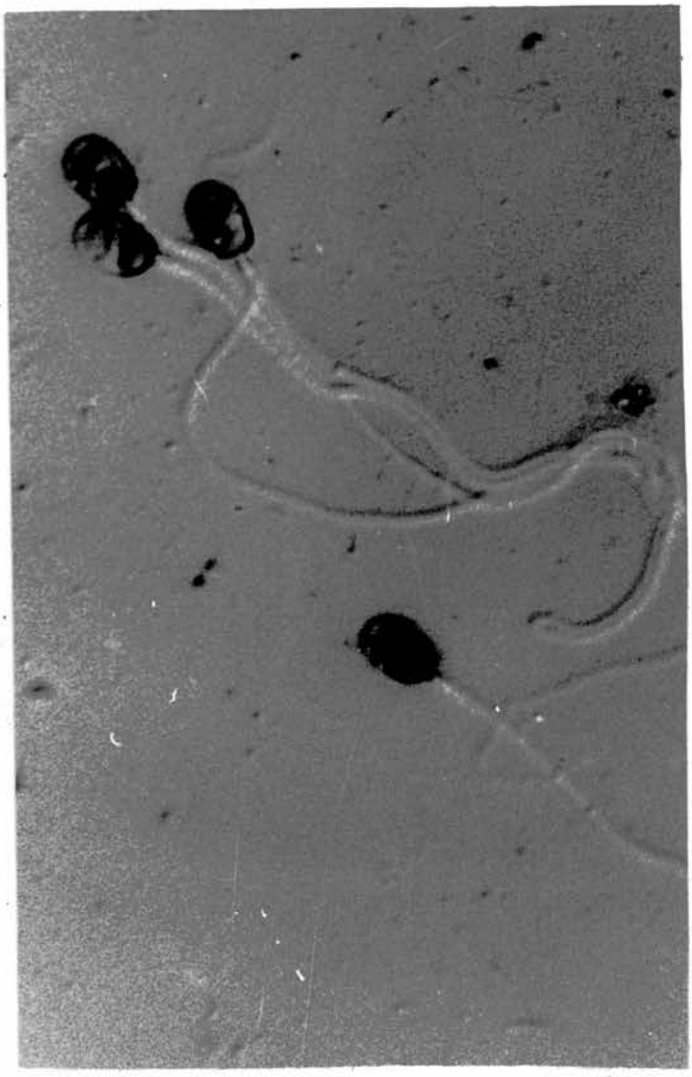


Fig.9.3. Spermatozoa of Pongo pygmaeus viewed with Nomarski inferference. Scale bar 10mμ.

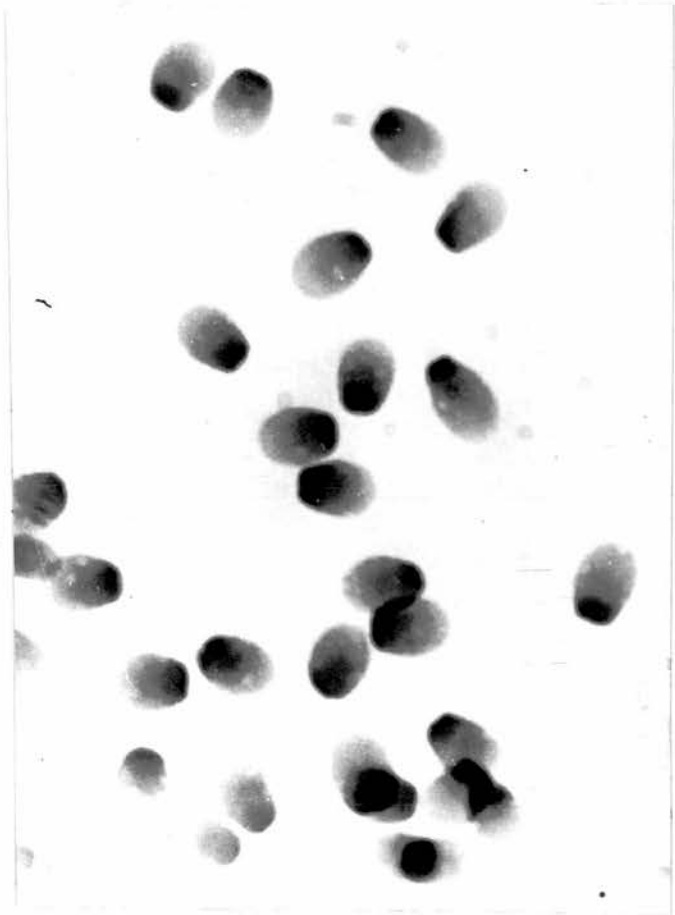


Fig.9.4. Spermatozoa of Pongo pygmaeus with Papanicolaou staining. Scale bar 10mμ.

(normal) spermatozoon found in the human ejaculate. With low magnification and with Nomarski interference a clear morphological pleomorphism is evident. In Fig.9.5, a large sized spermatozoon with two tails is present at the centre of the field; its head is practically twice the size of the majority of other spermatozoa seen. At a higher magnification it is possible to see that cells differ not only in size, but also in shape (Fig.9.6). A complete list of the types and frequencies of abnormal spermatozoa found in this and in other species is presented in Table 9.1. Observations were made on Papanicolaou stained preparations. It can be seen that the "normal" cell type in Gorilla gorilla accounts for approximately the same proportion of total spermatozoa as it does in man (approximately 70%), and that the most common abnormality found in the two species is the spermatozoon with a bizarre, irregularly shaped head. Thus, on morphological grounds, the spermiogram of man and Gorilla gorilla appear indistinguishable from each other. Fig.9.7 shows some human spermatozoa demonstrated by Nomarski interference, and the degree of pleomorphism present can be seen to be similar to that observed in Gorilla gorilla.

(C) Observations of spermatozoa with quinacrine staining.

The general observations made on sperm morphology and reported in the previous paragraphs were also observed with quinacrine fluorescence. In Pan troglodytes and Pan paniscus for example, the sperm heads appeared more intensely stained than the average human sperm head, and the level of fluorescence was highest at the region above the attachment of the tail (Fig.9.8b). The midpiece appeared wider and thicker than in human spermatozoa, and stained as intensely as/

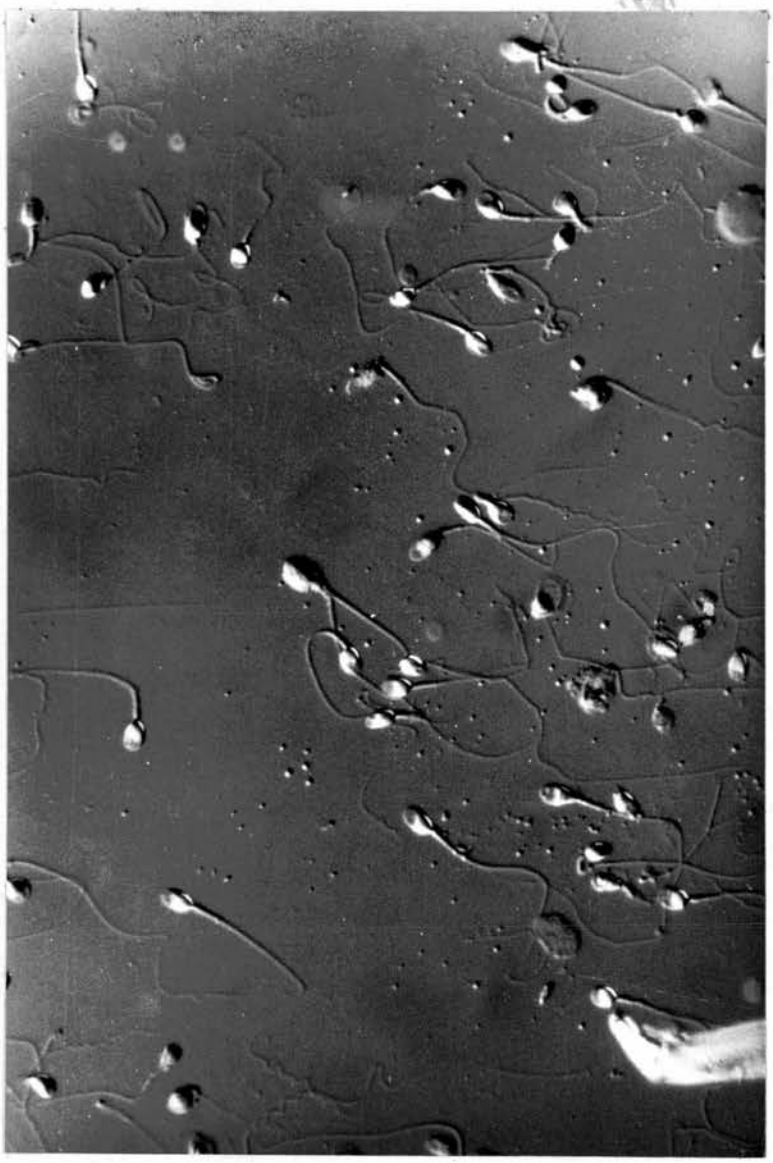


Fig.9.5. Spermatozoa of Gorilla gorilla viewed with Nomarski interference. Note the morphological pleiomorphism. Scale bar 10mμ.

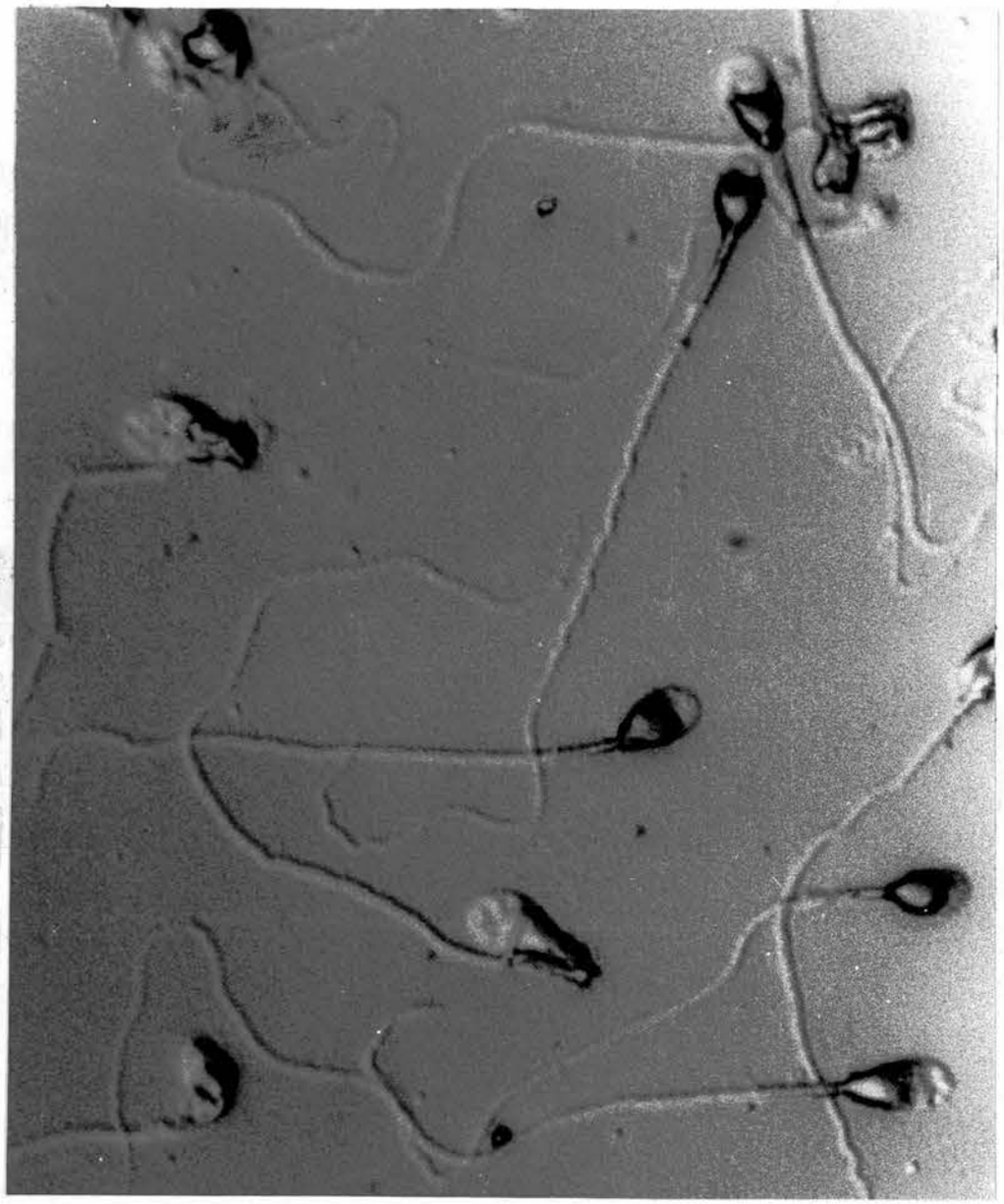


Fig.9.6. Spermatozoa of Gorilla gorilla views with Nomarski interference at a higher magnification. Scale bar 10mμ.

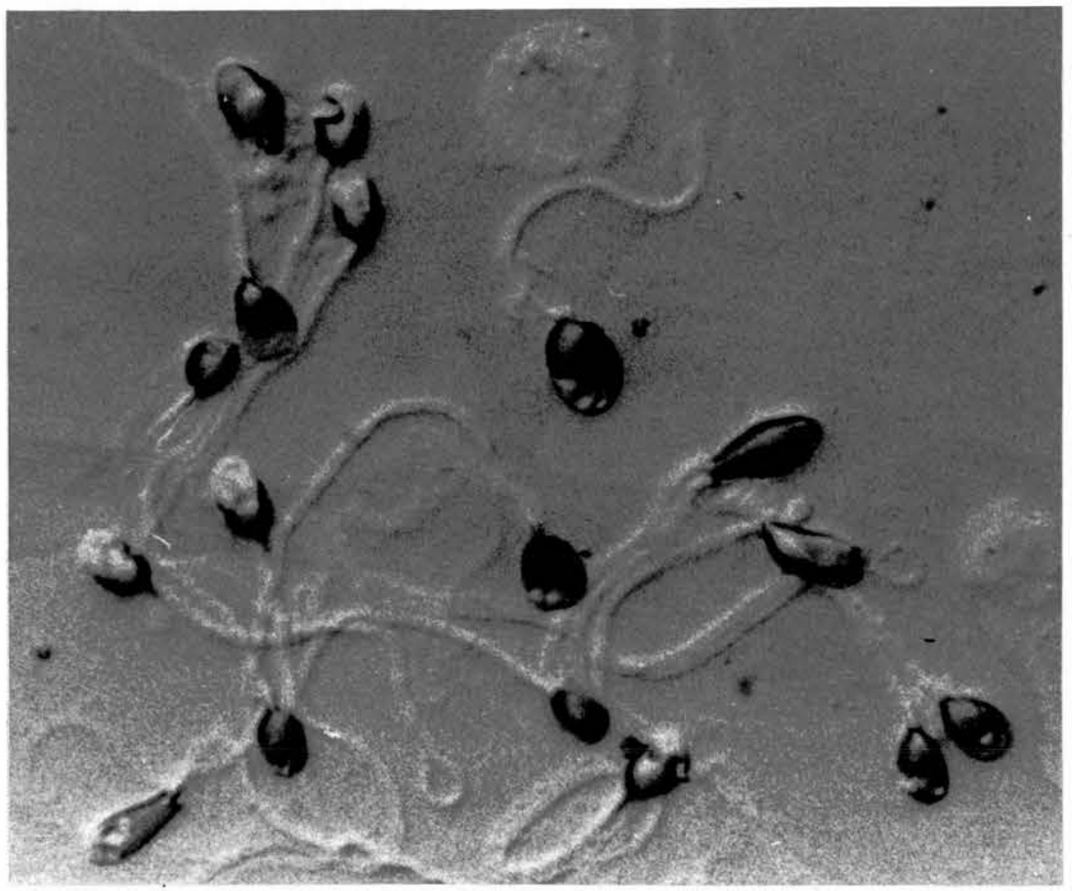


Fig.9.7. Human spermatozoa viewed with Nomarski interference. Note the morphological pleiomorphism. Scale Bar 10mμ.

TABLE 9.1

MORPHOLOGY OF SPERMATOZOA OF MAN AND THE GREAT APES

| | Homo sapiens (4 individuals) | Pan troglodytes (3 individuals) | Pan paniscus (1 individual) | Gorilla gorilla (2 individuals) | Pongo pygmaeus (2 individuals) |
|---|---|---|---|---|---|
| "Normal", i.e. Modal | 73.0 | 95.5 | 98.0 | 71.0 | 98.5 |
| "Abnormal": Large head | 2.0 | 0.3 | - | 2.3 | - |
| : Small head | 0.5 | - | 0.5 | 0.8 | - |
| : Tapered head | 0.3 | 0.2 | - | 1.0 | - |
| : Dense-staining | 0.6 | 0.2 | 0.5 | 4.5 | - |
| : Vacuolated head | 2.4 | 3.0 | 1.0 | 1.3 | 0.3 |
| : Irregularly shaped | 18.4 | 0.2 | - | 16.0 | 0.8 |
| : Multiple heads | 0.5 | - | - | 0.5 | - |
| : Abnormal midpiece | - | 0.3 | - | 0.5 | - |
| : Cytoplasmic droplets | - | - | - | 0.3 | - |
| : Immature cells | 2.3 | 0.3 | - | 2.0 | 0.5 |
| TOTAL | 100.0 | 100.0 | 100.0 | 100.2 | 100.1 |

(Two hundred cells were scored per individual and all results of Table 9.1 are expressed as percentages)

as the sperm head (Fig.9.8d). In human spermatozoa the same region is usually negatively stained under these conditions. Distinct brilliant fluorescent spots (F bodies) similar to those observed in human spermatozoa were observed. Their position did not show any preferential location, although those in the boundary between the dense area of the head and the more distal region below the acrosomic cap were better contrasted and were easier to photograph (Fig.9.8b). The number of cells with F bodies and the number of F bodies per cell are shown in Table 9.2. It can be seen that a much higher percentage of sperm showing an F body was observed in Pan paniscus than in any other species including Pan troglodytes.

In Gorilla gorilla a wide variation of head shape and size was evident as it was with Nomarski interference and with Papanicolaou staining (Fig.9.9). The head was usually intensely stained, but the midpiece was negatively stained as it usually is in human spermatozoa under similar conditions. Distinct F bodies were identified (Fig. 9.10 a to e) but their position appeared to be random in the sperm head. They could be clearly observed against the pale background below the acrocentric cap (Fig.9.10a)in the boundary between the dense staining area of the head and the pale distal end (Fig.9.10c), and sometimes below the boundary between these two regions (Fig.9.10b). Spermatozoon heads with two distinct F bodies were common (Table 9.2), and here again they seemed to be randomly located in any region of the head (compare Fig.9.9 d and e). Spermatozoa with more than two F bodies were also found, although they were usually in different planes of the head which made them impossible to photograph.

In Pongo pygmaeus the intensity of fluorescence in the spermatozoal head was pale, and decreased gradually from the centre of/

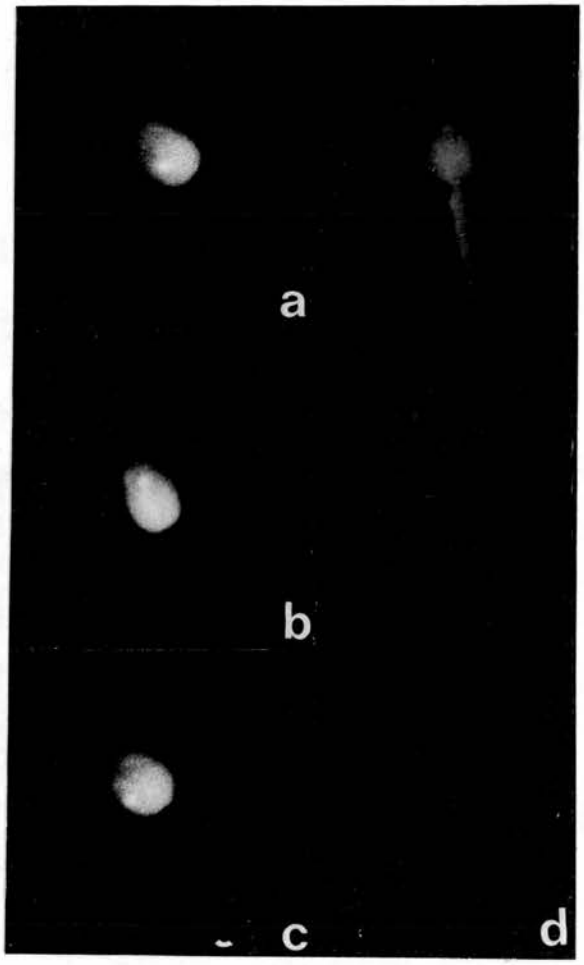



Fig.9.8. (a to d). Spermatozoa of Pan troglodytes with quinacrine fluorescence staining. Note visible F bodies.

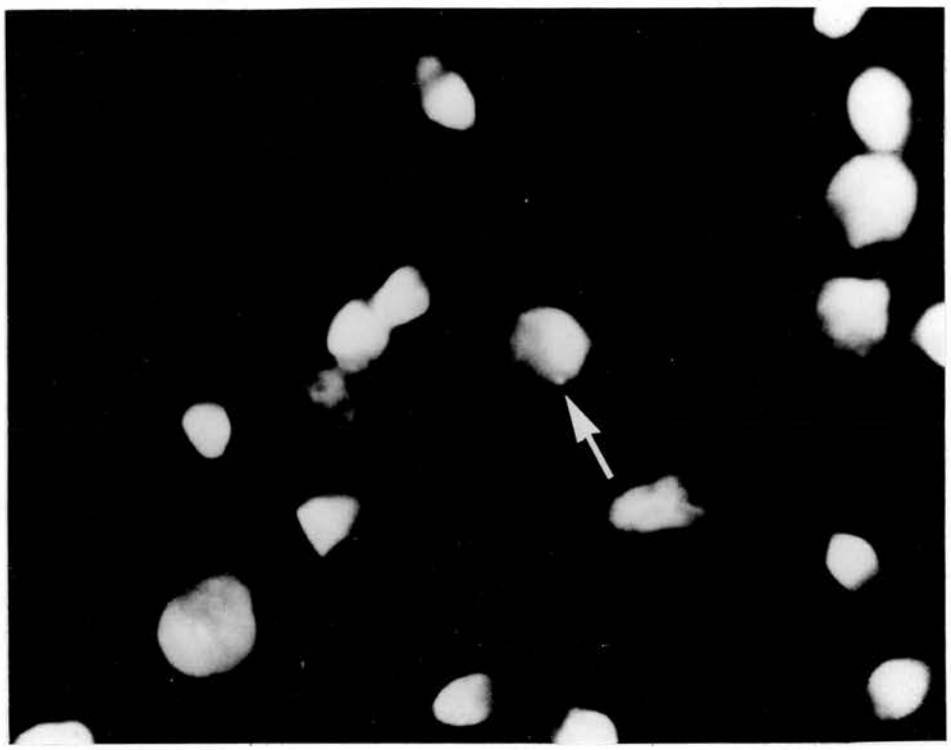


Fig.9.9. Spermatozoa of Gorilla gorilla with quinacrine staining. Note the variable size and shape of sperm heads. Arrow points to an F body.

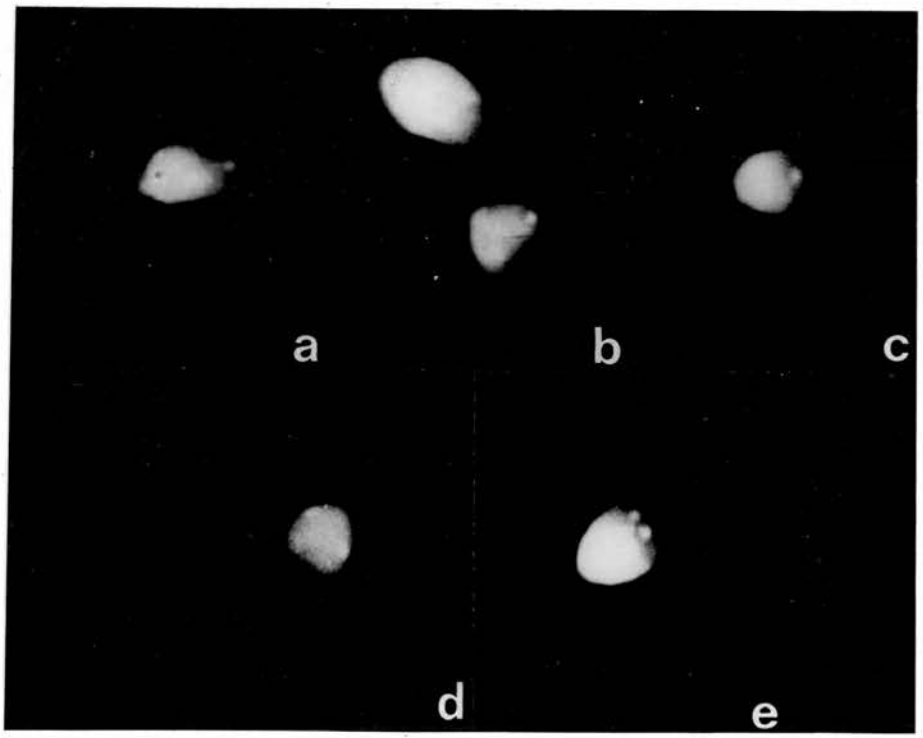



Fig.9.10, (a to e). F bodies in the spermatozoa of Gorilla gorilla.

TABLE 9.2

DISTRIBUTION OF F BODIES IN THE SPERMATOZOA OF THE GREAT APES.

| | Pan Troglodytes | | | | | Pan paniscus | Gorilla gorilla | | Pongo Pygmaeus | |
|---|---|---|---|---|---|---|---|---|---|---|
| Number of F bodies | 1 | 2 | 3 | 4 | 5 | 1 | 1 | 2 | 1 | 2 |
| 0 | 419(83.8%) | 68(34.0%) | 156(78.0%) | 264(52.8%) | 286(57.2%) | 5(2.50%) | 203(40.6%) | 185(37.0%) | 100(100%) | 100(100%) |
| 1 | 73(14.6%) | 74(37.0%) | 31(15.5%) | 194(38.8%) | 183(36.6%) | 118(59.0%) | 181(36.2%) | 197(39.4%) | - | - |
| 2 | 8( 1.6%) | 52(26.0%) | 13( 6.5%) | 42( 8.4%) | 30( 6.0%) | 63(31.5%) | 93(18.6%) | 104(20.8%) | - | - |
| 3 | - | 5( 2.5%) | - | - | 1( 0.2%) | 14( 7.0%) | 22( 4.4%) | 14( 2.8% | - | - |
| 4 | - | 1( 0.5%) | - | - | - | - | 1( 0.2%) | - | - | - |
| TOTAL .. .. .. | 500(100%) | 200(100%) | 200(100%) | 500(100%) | 500(100%) | 200(100%) | 500(100%) | 500(100%) | 100(100%) | 100(100%) |
| Cells with even numbers of F bodies 0,2,4 | - | 121(60.5%) | - | 306(61.2%) | 316(63.2%) | - | 297(59.4%) | 289(57.8%) | - | - |
| Cells with odd numbers of F bodies (1 and 3) | - | 79(39.5%) | - | 194(38.8%) | 184(36.8%) | - | 203(40.4%) | 211(42.2%) | - | - |
| Chi-square (df = 1) | - | $X_1^2 = 8.82$ | - | $X_1^2 = 25.08$ | $X_1^2 = 34.84$ | - | $X_1^2 = 17.67$ | $X_1^2 = 12.16$ | - | - |
| Probability | - | $p < 0.01$ | - | $p < 0.0001$ | $p < 0.0001$ | - | $p < 0.0001$ | $p < 0.001$ | - | - |

of the head to the periphery. The midpiece was negatively stained. No visible F bodies were detected.

(D) Chromosome constitution of the animals from which semen samples were obtained.

Unfortunately it was impossible to study the chromosomes of all animals from which semen samples were obtained. Of the five specimens of Pan troglodytes chromosome studies were made in specimen Nos. 4 and 5 which correspond to animal I.4 and I.5 Chapter 2, (Table 2.5). The only specimen of Pan paniscus available corresponds to animal I.1 in Chapter 2, Table 2.5, and its Q-band karyotype is shown in Fig.9.12. Of the two specimens of Gorilla gorilla studied, one (No.2) was karyotyped and this corresponded to animal I.5 in Chapter 3, Table 3.4. Data on these animals showed how many brilliant polymorphisms were present in the chromosomes of each, and permitted us to compare these with the number of F bodies observed in the spermatozoa. The two specimens of Pongo pygmaeus studied correspond to animals S.I.16 and S.I.17, the former a heterozygous carrier for a rearranged chromosome 9 and the latter a homozygous carrier for the same rearranged chromosome 9 (Chapter 4, page 50) Both animals produced morphologically normal spermatozoa which was regular in size and shape (Table 9.1).

(E) Discussion.

The morphological attributes of spermatozoa within the Hominidae show considerable variation between some species, and remarkable similarities between other species. Spermatozoa of both species of chimpanzee/

chimpanzee are practically identical,but since they are the two most closely related species within the Hominidae, their similarity in spermatozoal morphology is perhaps not surprising. Spermatozoa of man and Gorilla gorilla are remarkably similar in several respects. One is the fact that the modal type of sperm in these two species is practically identical, the other is that it occurs with similar frequency in the ejaculate. The third is that both species demonstrate a remarkably similar range of pleiomorphic forma. The number of individuals studied is admittedly too small to allow general conclusions to be drawn, but it is important to mention that all the individual men and gorillas from which semen samples were obtained were phenotypically normal and of proven fertility. It is interesting to observe that when spermatozoa of different animal species are compared (see Chapter 8), remarkable differences are observed, although some characteristics may be found in common in phylogenetically related taxa. Bedford (1974), for example, has pointed out that the spermatozoa of man, Pan troglodytes and Hylobates lar exhibit certain characteristics which differentiate them from spermatozoa of the Cercopithecoidea. These include a less bulbous acrosome, the minimization or the absence of a perforatium, the shorter nucleus which is flattened in the dorso ventral plane, and a reduction in the length of the midpiece and numbers of mitochondria. However, other dimensions vary considerably between species and are not necessarily correlated with an apparent successful adaptation, but rather seem to have appeared as a consequence of genetic drift which accounts for the morphological differences observed between very closely related species such as man, Pan troglodytes and Pongo pygmaeus. If we were to construct a phylogenetic tree of the Hominidae/

Hominidae based on spermatozoal morphology, the most primitive spermatozoon would probably be that of Pongo pygmaeus since it shows some features in common to the Cercopithecoidea, such as a very prominent post acrosomic margin. The next branch will open in a dichotomy, one fork including the genus Pan with identical regular shaped and sized spermatozoa, and another the genera Homo and Gorilla with pleiomorphic spermatozoa. The significance of spermatozoal pleiomorphism is still unknown in man and in Gorilla gorilla. Bedford (1974) has postulated that the observed morphological pleiomorphism and other attributes which differentiate human spermatozoa from those of other species may result from clothing induced hyperthermia, but the finding that the spermatozoa of Gorilla gorilla exhibits a similar pleiomorphism, and that these animals are maintained in unheated cages at the Yerkes Regional Primate Centre makes it unlikely that testicular hyperthermia may account for the observed morphological pleiomorphism of human spermatozoa. It would be of interest to observe if the spermatozoa of Gorilla gorilla exhibited the same irregular degree of swelling observed in human spermatozoa by Bedford et al (1972) using Cleland's Reagent. This might provide a better understanding of the nature of pleiomorphic spermatozoa, and verify whether such pleiomorphism is related to the fact that spermatozoa of different degrees of maturation are mixed in the epididymis and released into the ejaculate as suggested by Bedford et al (1972).

The detection of F bodies in the spermatozoa of two species of chimpanzee and the gorilla and their absence in the orangutan was not unexpected in view of the quinacrine banding patterns of these species, (Chapters 2, 3 and 4). In Fig.9.11 the brilliant fluorescent regions/

regions of man, Pan troglodytes and Gorilla gorilla are compared; Fig.9.11 shows the Q-band pattern of Pan paniscus. From this comparison it can be seen that in man, the brilliant fluorescent regions in the autosomes are rarely as large in size as that in the distal tip of the Y chromosome, and this might be a reason why these regions are unlikely to show as an F body in spermatozoa. The brilliant fluorescent region of the Y chromosome in man is usually visible, although in somewhat less than 50% of spermatozoa (Pearson and Bobrow, 1970; Summer et al, 1971b; Pawlowitski and Pearson, 1972; Geraedts and Pearson, 1976), since it has been estimated that the visibility of the Y chromosome in spermatozoa is below 100% in man (Beatty, 1977). In Pan troglodytes and Pan paniscus, where the Y chromosome is pale with fluorescence, the F bodies must correspond to autosomal regions. In Gorilla gorilla where both the Y chromosome and the autosomes show brilliant fluorescence, and the size of the autosomal regions may sometimes be as large in size as that of the Y chromosome (Fig.9.11) we would expect that both the Y chromosome and some autosomal regions would show as visible F bodies. From previous studies on the chromosomes of some of the animals from which semen samples were obtained we know that animal No.4 of Pan troglodytes carried 7 brilliant polymorphisms in its chromosome complement whereas No.5 carried 10 brilliant polymorphisms, as did the specimen of Pan paniscus (Fig.9.12). Specimen No.2 of Gorilla gorilla carried 15 autosomal brilliant polymorphisms and a brilliant Y chromosome. The distribution of F bodies found in these animals shows that in Pan troglodytes and in Gorilla gorilla the observed distribution does not coincide with our expectations. A clear example is the carrier of 10 brilliant polymorphisms in Pan troglodytes, all the regions being/

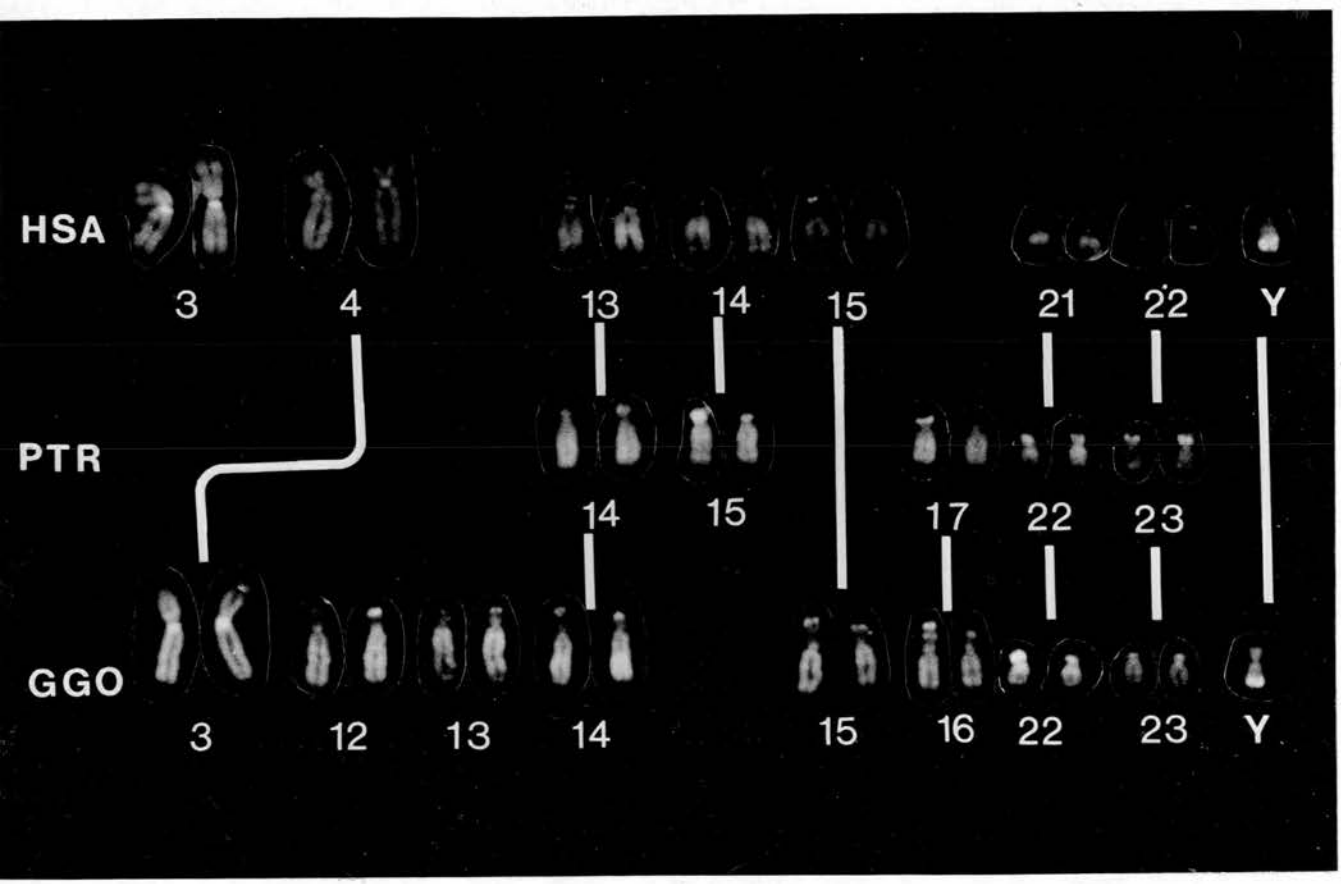



Fig.9.11. Comparison of the brilliant fluorescent regions of man (HSA), Pan troglodytes (PTR) and Gorilla gorilla (GGO). Vertical lines denote homologous chromosomes between species.

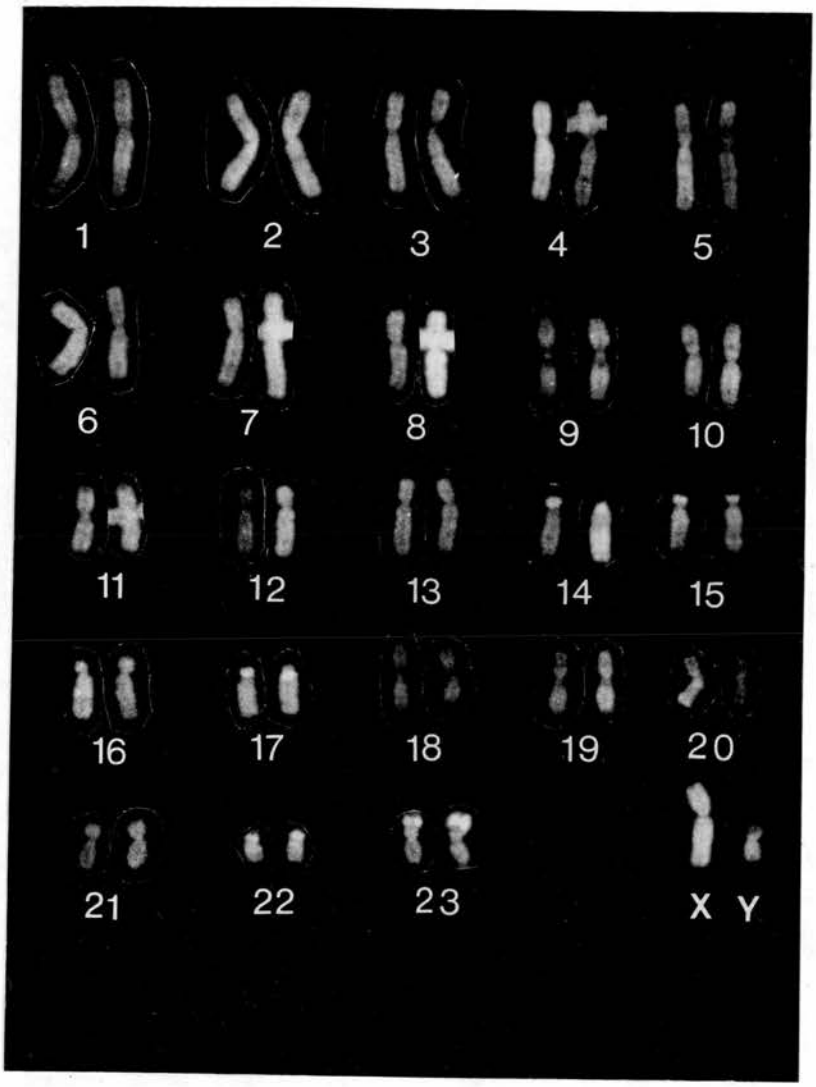



Fig.9.12. Q-band karyotype of Pan paniscus. Note the large brilliant regions of chromosome 23.

being of approximately the same size (see Chapter 2, Table 2.5). Should all these regions be visible in spermatozoa we would expect to find all spermatozoa with 5 F bodies. Should any single one of these regions be always visible, all spermatozoa would at least show 1 F body. However, more than half (57%) of the spermatozoa scored in this animal showed no F body, and the maximum number of F bodies scored was 3. The animal who is a carrier of 7 brilliant polymorphisms (No.4) should at least show 2 F bodies per spermatozoon and a maximum of 5 F bodies if all the brilliant regions of its autosomes were always visible in spermatozoa (Chapter 2, Table 2.5). However, slightly above half of the spermatozoa scored in this animal showed no F body (Table 9.2). In the male gorilla (No.2) there are 15 brilliant polymorphisms in the autosomes and a brilliant region at its Y chromosome (Chapter 3,Table 3.4) but 37% of its spermatozoa showed no F body (Table 9.2). These results point to the fact that many brilliant fluorescent regions are not visible as distinct F bodies in spermatozoa of these animals. However, in other animals in which approximately half or more of the spermatozoa show at least 1 F body, the possibility must be tested that at least one brilliant region may show constantly and this will be commented upon later.

In the other three specimens of Pan troglodytes and in one of Gorilla gorilla from which blood samples were not obtained, we have no information on the number of brilliant polymorphisms which they may carry. However, it is useful to take into consideration what we have previously estimated as the average number of brilliant polymorphisms in these species, and see what distribution of F bodies we might expect from such estimation. In Chapter 2 we have estimated that the average number of brilliant polymorphisms in Pan troglodytes was

8.87/

8.87, so that we would expect that if these regions were all equally visible a minimum number of 4 F bodies per spermatozoon would be found. In Gorilla gorilla the number of autosomal brilliant polymorphisms was estimated as 14.9 (Chapter 3) so that we would expect that if all these regions were equally visible, as well as that of the Y chromosome, a minimum of 7 F bodies would be observed. The observed distribution, however, shows a completely different result from that expected. In Pan troglodytes the distribution found in specimen Nos.1 and 3 shows that less than 25% of spermatozoa show F bodies, thus indicating that none of the brilliant fluorescent regions in the chromosomes of these animals is capable of showing constantly as an F body in spermatozoa. In specimen No.2 of Pan troglodytes, as in both gorillas the incidence of spermatozoa with no F body ( 0 class) is below 50% so that it could be possible that one brilliant region may be constantly showing in spermatozoa. This could also be the case of specimen Nos.4 and 5 of Pan troglodytes in which the incidence of spermatozoa with no F body (0 class) was slightly above 50%. If there were any single heterozygous brilliant region in any animal capable of showing constantly as an F body (call it $F_1$, say) we would expect that 50% of spermatozoa would show it. Assuming all other F bodies showed independently of $F_1$, then the number of spermatozoa containing $F_1$ and $n$ other F bodies should be the same as the number not containing $F_1$, but containing other F bodies. Since this should hold for all values of $n$, the number of spermatozoa containing 0, 2, 4... F bodies (even class) should equal the number containing 1, 3, 5... F bodies ( odd class). Since the even class is significantly greater ($X^2$ test) ($P < 0.01$) than the odd class the original hypothesis can therefore be ruled/

ruled out. When this test is applied to animals showing an incidence of the O class near 50% and below this value, it was shown that none of these animals has a brilliant fluorescent region capable of showing constantly in spermatozoa. This observation is of interest in both species tested, Pan troglodytes and Gorilla gorilla in which the estimated number of brilliant polymorphisms has been found to be significantly higher than in man. In the case of Gorilla gorilla it implies that the brilliant Y chromosome in this species cannot be visualized in spermatozoa as constantly as the human Y chromosome, since the former has a lower visibility than the latter. In man, the F body is a useful indicator of the Y chromosome since its incidence in spermatozoa is slightly below 50%, although Beatty (1977) has estimated that its visibility is only 83%. In Gorilla gorilla, on the other hand, other factors apart from a lower visibility make the F body unreliable as an indicator of the Y chromosome, and this is the fact that some autosomal regions may also be visualized as brilliant spots. This is evident when analysing the distribution of spermatozoa with more than one F body. In man, the incidence of spermatozoa with two F bodies is only 1.25% (Sumner et al, 1971); in the two gorillas studied it was approximately 19%. In man, the presence of two F bodies was explained as resulting from non disjunction of the Y chromosome in the second meiotic division producing YY bearing spermatozoa. However, it has been considered that the two F bodies in a human sperm may be produced if a Y bearing spermatozoa were incompletely condensed rather than by non disjunction of the Y chromatids (Sumner and Robinson, 1976). Thus, it is probable that the high incidence of spermatozoa with two F bodies in the gorilla does not represent YY bearing spermatozoa, but is attributable/

attributable to the fact that some autosomal regions are also visible. The existence of spermatozoa with more than two F bodies supports this hypothesis.

Finally, it is important to analyse the distribution of F bodies in the one male specimen of Pan paniscus. As illustrated in Fig.9.12 this animal showed 10 autosomal polymorphisms, those being larger in size on chromosomes 14 and 23. The distribution obtained in Table 9.2 shows that 97.5% of spermatozoa showed at least one F body and the maximum number of F bodies observed was 3. This suggests that one homozygous brilliant region must be frequently visible, but no more than two other regions are visible at all. The distribution observed in Table 9.2 is coincident with the assumption that one brilliant autosomal pair shows 95% visibility and two brilliant homozygous pairs show 25% visibility each. Table 9.3 shows an estimation for 3 different values of visibility for one homozygous brilliant region, and shows that the value of 95% gives the best fit for the observed distribution.

| F | N | 0.94 | 0.95 | 0.96 |
|---|---|---|---|---|
| 0 | 5 | 6.75 | 5.62 | 4.5 |
| 1 | 118 | 110.25 | 110.62 | 111.0 |
| 2 | 63 | 71.25 | 71.87 | 72.5 |
| 3 | 14 | 11.75 | 11.87 | 12.0 |
| | 200 | | | |

Thus, in this animal the F body is a useful indicator of one autosomal region which probably corresponds to that of chromosome 23 which is larger in size than any other. It is also important to remark that F bodies in these animals appeared to be randomly located in the spermatozoal head. This finding supports the observation of

Geraedts/

Geraedts and Pearson (1976) after staining human spermatozoa by the G-11 technique and with quinacrine fluorescence. This combined procedure allows the identification of chromosome 9 and the Y chromosome, and both of these were found to be randomly located. A similar conclusion was reached by us (Seuanez et al, 1976c) using in situ hybridisation to cRNA III in human meiotic preparations coupled with quinacrine fluorescence. These studies gave no indication that chromosomes in human spermatozoa might have a constant orientation.

(F) Summary

(1) The morphology of the spermatozoa of man and of the great apes has been compared and this study has indicated that man and the gorilla show pleiomorphic spermatozoa, whereas in the other species spermatozoa are regularly shaped.

(2) The modal (normal) spermatozoa of man and gorilla were identical, occurring with a similar frequency in the ejaculate. Moreover both species showed an identical range of pleiomorphic forms.

(3) The morphology of the spermatozoa of Pan troglodytes was identical to that of Pan paniscus, but distinct from all other species.

(4) The degree of pleiomorphism observed in the gorilla makes it unlikely that clothing induced hyperthermia is the cause of sperm pleiomorphism found in the human ejaculate.

(5) Visible "F" bodies were identified in the spermatozoa of Pan troglodytes, Pan paniscus and Gorilla gorilla, but not in Pongo pygmaeus.

(6) The "F" bodies in Pan troglodytes and Pan paniscus represent brilliant autosomal regions whereas in the gorilla they probably rperesented both autosomal regions and the Y chromosome.

(7) In Pan troglodytes and Gorilla gorilla none of the brilliant fluorescent regions was capable of showing consistently as an "F" body in spermatozoa. Thus, the "F" body is a poor indicator of the Y chromosome in the gorilla, contrary to what is found in man.

(8)/

(8) In the specimen of Pan paniscus one brilliant autosome region showed consistently in the spermatozoa.

# CHAPTER 10: DNA CONTENT OF SPERMATOZOA OF MAN AND THE GREAT APES.

## (A) Introduction.

The study of human spermatozoa has always been a matter of interest to understand some of the intriguing problems in human reproduction. One of the questions is whether the production of pleomorphic spermatozoa implies that a large proportion of them are genetically defective. There exists the possibility that this might be the case, since one out of every two conceptions has been found to be chromosomally abnormal (Boue et al, 1975). Moreover, Beatty (1974) has estimated that 40% of triploid fetuses are produced by diandry (fertilization of an egg by a sperm containing a diploid chromosome complement). More recently, it has been proposed that hydatidiform moles are produced when an inactive egg is fertilized by a diploid spermatozoon (Kajii and Ohama, 1977). However, the relationship between spermatozoal morphology and DNA content is not clear. The fundamental problem is whether the genome of the spermatozoon is capable of expressing itself in the spermatozoal phenotype thus affecting either its morphology, motility, or any specific function which could be vital for its reproductive performance. The alternate possibility is that genetic control of the spermatozoon is mediated by the diploid soma and by the premeiotic cells from which spermatozoa are derived.

The production of morphologically abnormal spermatozoa is significantly increased in infertile men who are carriers of an abnormal chromosome complement, probably because chromosomally unbalanced gametes result from different types of meiotic segregation (Chandley et al/

et al, 1975). However, it is not known whether this increase is due to an increase in aneuploid spermatozoa, or whether it has resulted from an overall disturbance of spermatogenesis determined by the soma of the carrier. In general, the view is that the capacity of a spermatozoon to fertilize is not influenced by its own haploid genotype (Beatty, 1975). One notable exception to this rule is provided by the T-locus in mice (Braden, 1958), in which there is evidence for post-segregational gene action. However, in all other situations so far studied in the mouse (Ford, 1972) and in Drosophila melanogaster (Lindsley and Grell, 1969) the development and function of the spermatozoon appears to take place entirely under the influence of the diploid genome of the germ line. In situations in which diploid spermatozoa are produced there appears to be some kind of relationship between head size and DNA content. In the rabbit, Carothers and Beatty (1975) found that diploid spermatozoa could easily be recognized by their larger size. A similar situation seems to occur in man (Sumner, 1971). However, not all large spermatozoa are necessarily diploid, either in the rabbit or in man.

Another important factor that makes the spermatozoon unique is that its DNA content is apparently stable and genetically inactive. DNA synthesis in the meiotic cycle of both plants and animals occurs at the S-phase - prior to the meiotic prophase (Taylor and McMaster, 1954; Monesi, 1962; Heller and Clermont, 1964; Hochereau, 1967). Although a small additional DNA synthesis occurred during the prophase of the first meiotic division in plants (Hotta et al, 1966), the mouse (Hotta et al, 1977), and also in man (Lima de Faria, et al, 1968), all the DNA synthesis is completed before metaphase of the first meiotic/

meiotic division. Thus, the amount of DNA which is contained in the spermatids and spermatozoa will only depend on how chromosomes segregate and divide during meiosis. Since the amount of DNA does not increase or decrease in spermiogenesis (the maturation of spermatids into spermatozoa) the DNA content of spermatids and spermatozoa remains constant and does not fluctuate (Monesi, 1971).

This unique property of spermatozoal DNA has encouraged many investigators to make estimations of the content of DNA in spermatozoa. The methods used for spermatozoa or somatic cells are based on several different principles and techniques. The most accurate estimations are those in which measurements are made on individual cells, since estimations on samples or on cell suspensions are biased by the fact that the exact number of cells can never be determined accurately. In general, DNA estimations on individual cells are based on two principles. One depends on the property of substances to absorb light of a known wavelength, so that an estimation of the amount of the substance can be made by measuring the amount of absorbed light (Absorbance). DNA for example, absorbs UV light, and this method has been used for microdensitometric analysis of unstained cellular preparations. Unfortunately RNA also absorbs UV light and this method therefore requires the elimination of RNA with RNA-se. However, in spermatozoa the amount of RNA is insignificant (Leidl et al, 1973). DNA does not absorb visible light, but if suitably stained it can be made to do so. For quantitative estimation, however, the stain must be specific for DNA, so that the amount of stain bound to a cell is positively correlated with the amount of DNA. Both these properties are possessed by the Schiff reagent used in the Feulgen reaction. A second approach to this problem/

problem is to estimate DNA indirectly by measuring the total dry mass (TDM) of spermatozoa. This estimation provides information on the total amount of substance (protoplasm) in the sperm head but not of its nature or composition. It is based on the fact that light, passing through an unstained object with a higher refractive index than the background, suffers a wavelength retardation in relation to the light passing through the background (Ross, 1967). The amount of phase retardation (or optical path difference) will depend on the refractive index of the object, which in turn will depend on its total dry mass. This method, although it only provides an indirect estimate of the DNA content of spermatozoa, is technically more reliable, and this will be commented on in a special section.

Some early estimations of DNA content in human spermatozoa were done with Feulgen stained cells. Leuchtenberger et al (1953, 1956) compared the DNA content of normal and infertile men and claimed that men with infertility problems had a lower DNA content than men with normal fertility. Fluctuations in DNA content were also greater in infertile men when spermatozoa from different semen samples were compared, but such changes do not necessarily indicate any chromosomal aberration in the spermatozoa of infertile men; Gledhill (1970) has suggested that such diminutions in Feulgen values in infertile men could be due merely to biochemical immaturity of proteins associated with spermatozoal DNA rather than to a lower DNA content. The interest in estimating the DNA content of human spermatozoa increased when it became possible to differentiate X bearing from Y bearing spermatozoa by differential fluorescent staining (Barlow and Vosa, 1970). A combined technique for identifying/

identifying Y bearing spermatozoa with quinacrine fluorescence and measuring the DNA content of the same cells after Feulgen staining, permitted the identification of two populations of spermatozoa: one with a greater DNA content (the X bearing), and another with a smaller DNA content (the Y bearing) (Sumner et al, 1971b; Evans, 1972; Geraedts and Pearson, 1976). In between both groups there was a third group of spermatozoa which showed two fluorescent spots (F bodies) which were believed to correspond to YY spermatozoa. This kind of spermatozoon showed Feulgen values greater than those for Y bearing spermatozoa, but lower than the values for the X bearing group. However, it was later postulated that the presence of two F bodies did not necessarily imply two Y chromosomes (Sumner and Robinson, 1976), but could result from an incomplete packaging of chromatin inside the head of the spermatozoon. This latter study was carried out with an integrating interferometer and DNA was estimated indirectly through estimations of total dry mass of the head of the spermatozoon, thus producing more accurate information than previous measurements by the Feulgen technique.

Preliminary observations on the spermatozoa of man's closest living relatives, the great apes, have been reported (Martin et al, 1975; Seuanez et al, 1976d), and these studies have shown that there is a remarkable morphological pleiomorphism in the gorilla as there is normally in human spermatozoa, in contrast with the morphologically uniform spermatozoa of the chimpanzee and the orangutan. It was obviously of interest to look at the DNA content of the spermatozoa of the great apes and compare it with that of man, and this study will be presented in this chapter.

(B)/

(B) Material and Methods.

The source of material and preparation of slides was described in the Appendix (Section III). DNA estimations were made on spermatozoa of individuals of proven fertility, as follows: 4 men, 3 chimpanzees (Pan troglodytes), 1 pygmy chimpanzee (Pan paniscus), two orangutans (Pongo pygmaeus) and two gorillas (Gorilla gorilla). The number of individuals is admittedly small, but it was impossible to obtain material from a larger number of these very rare animals. In general, a total of 50 cells was measured per individual using two slides (25 measurements in each slide), although for the gorillas 100 measurements per individual were made (50 per slide per each animal). Spermatozoa were measured at random; thus in man and the gorilla where a large proportion of pleiomorphic spermatozoa are found, no selection took place, and spermatozoa of all shapes and sizes were measured. Measurements were carried out on unstained preparations. The instrument used was a Vickers M-86 integrated microinterferometer (Vickers Instruments, York, England), which produces a result representing the optical path difference produced by the object with reference to background (its refractive index x its thickness). Results are expressed in arbitrary units; the area of the object to be measured (the spermatozoal head only) was selected with an electronic masking system. All measurements were made with a X 75, n.a. 1.1. water immersion objective, and all specimens were measured while immersed in distilled water.

(C) Evaluation of the method.

Since total dry mass measures the amount of protoplasm regardless of any specific component of substance, we tested whether the amount/

amount of dry mass was proportional to the DNA content of the spermatozoa. This test was made in spermatozoa from two individuals per species, except in the pygmy chimpanzee where only one individual was available. Ten cells were measured in each individual as already described for total dry mass, but the cells were photographed before scoring. They were then identified by numbers in the photographic print so that it was possible to know their individual TDM values. The slides were then treated with 5% trichloroacetic acid (TCA), at 90°C to extract the DNA. After extraction, the slides were washed in running tap water for one hour to remove the TCA, and the same spermatozoa were relocated, and each cell was measured again with the interferometer. Each measurement represented the residual dry mass after DNA extraction (DMAE) and each value was compared to the previously estimated value of TDM of the same cell. To ensure that all the DNA had been removed by the TCA extraction the slides were then stained by the Feulgen reaction. This was done by immersing the slides in a 5N HCL solution for 1½ hours at room temperature followed by 30 minutes of staining with Schiff reagent. After Feulgen staining the same spermatozoa were again relocated. The M-86 Vickers instrument was put in the microdensitometric mode and the spermatozoa were measured for residual DNA. Only when no residual DNA was found by Feulgen microdensitometry was the previous estimate of DMAE considered valid. We found that the minimum DNA extraction time for human spermatozoa was 15 minutes, while 30 minutes were required to extract all the DNA from the spermatozoa of the great apes.

Means of TDM and DMAE were estimated for each individual. The differences between the mean values of TDM and DMAE represents the mean/

mean value of the amount of DNA extracted by the TCA. This will be called DNA-DM. DNA-DM and TDM were then plotted in a graph and the estimated least-squares regression line was calculated (Fig. 10.1). The latter passes close to the origin, suggesting that proportional changes in TDM are identical to those of DNA-DM. The line should, of course, not be interpreted as a valid extrapolation beyond the range of the plotted points.

We have considered this procedure as the most reliable to estimate the DNA content of spermatozoa, even though DNA has been estimated indirectly (through dry mass), instead of by a more direct microdensitometric analysis of Feulgen stained spermatozoa. However, the stoichemistry of the Feulgen reaction depends on many variables such as the time of fixation of the material, the capacity of the stain to penetrate cells, the temperature and the time of hydrolysis, and the pH of the Feulgen reagent (Deitch, 1966). In the case of spermatozoa, the factor which usually makes Feulgen reaction unreliable for quantitative DNA estimations is the condensed state of the chromatin which makes it difficult for the stain to penetrate the spermatozoal heads (Gledhill, 1970). This is reflected by the fact that there is a greater variability of measurements with Feulgen stain than with UV light (Bouters et al, 1967), or dry mass estimations (Summer and Robinson, 1976) when measurements are made on the same cells.

The use of TCA to achieve DNA extraction is preferable to DNA-se on two grounds: (i) DNA-se is a large molecule and might have difficulties in penetrating the spermatozoal head, and (ii) DNA-se is a protein with a higher molecular weight than TCA. This would make the removal of DNA-se more difficult than TCA when washing with tap/

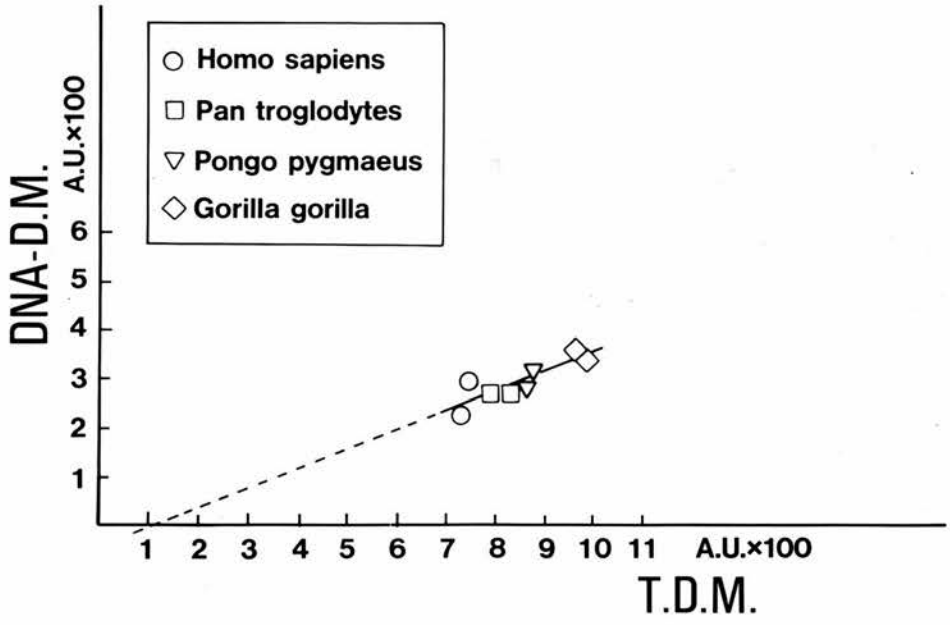



Fig.10.1. Least square-regression line between DNA dry mass (DNA-DM) and Total Dry Mass (T.D.M.). Each symbol in the graph represents mean values of one individual. (A.U. = arbitrary units.)

tap water, and residual amounts of DNA-se might affect measurements of DMAE.

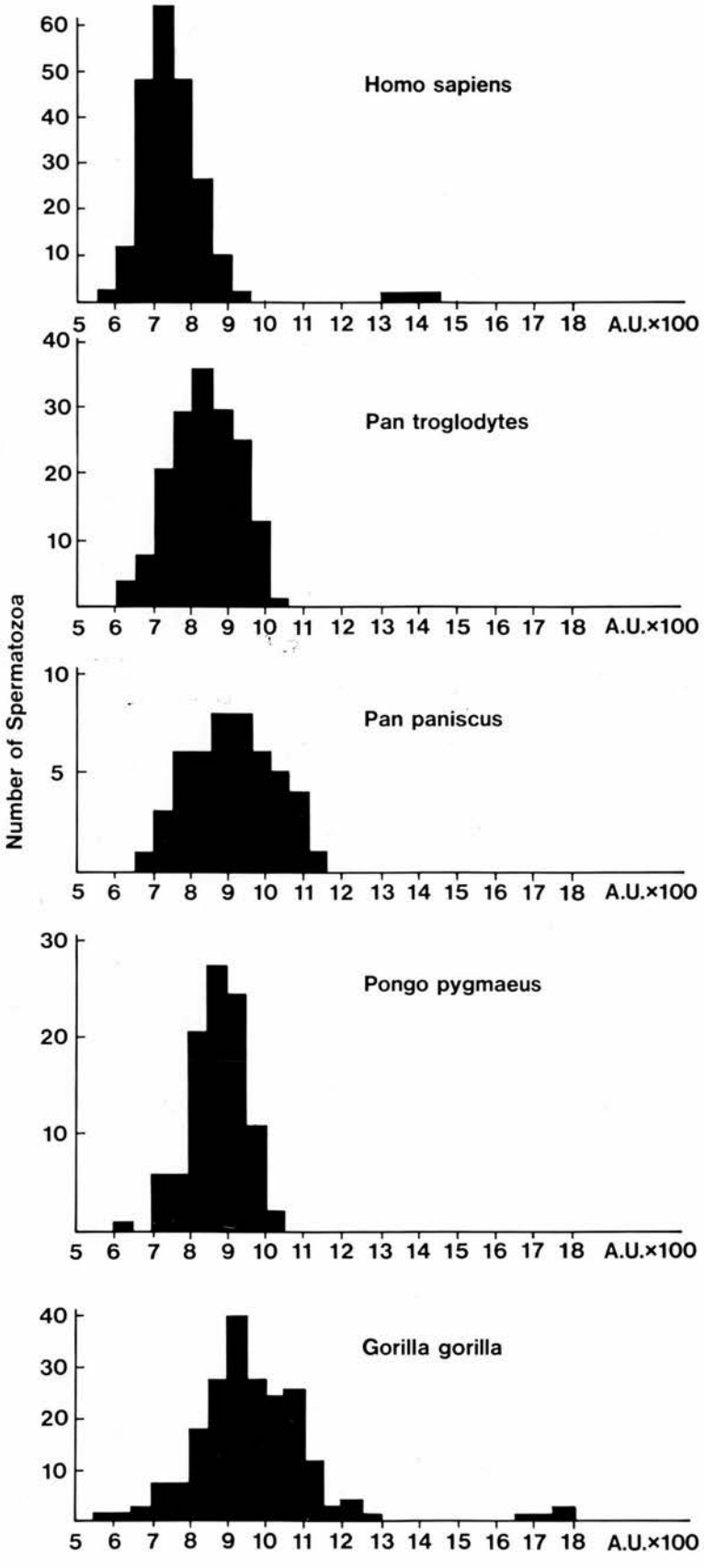
The use of Feulgen staining after DNA extraction was necessary to validate previous results of DMAE and to find an optimal extraction time for all the species under investigation.

(D) Results.

The distribution of values of total dry mass (TDM) of the spermatozoa of man and the great apes has been reported (Seuanez et al, 1977a) and is shown in Fig. 10.2. In man and the gorilla, the distribution was bimodal with two non-overlapping peaks, whereas in the chimpanzees and the orangutan it was unimodal. Using arbitrary units of dry mass the mean values of the major or peak in man was 730 compared to 930 in the gorilla. In both species the minor peak had a mean value approximately twice that of the major peak. These results are similar to those reported with Feulgen stained spermatozoa of man and the rabbit (Carothers and Beatty, 1975), and suggest that the groups represent haploid and diploid spermatozoa. The percentage of diploid spermatozoa in man and the gorilla was estimated in a larger sample; 21 diploids were found in 2000 human spermatozoa (1.05%) and 37 in 2478 gorilla spermatozoa (1.53%). The difference is not statistically significant. The percentage of diploid spermatozoa found in man was practically identical to that initially reported by Sumner (1971), although Carothers and Beatty (1975) have found a lower incidence of diploid spermatozoa in man, of approximately 0.5%.

Statistical/

Fig.10.2. Distribution of Total Dry Mass (T.D.M.) values for the spermatozoa of man and the great apes. Note the bimodal distribution in man and the gorilla.


Homo sapiens
Pan troglodytes
Pan paniscus
Pongo pygmaeus
Gorilla gorilla
Number of Spermatozoa
A.U.×100
5 6 7 8 9 10 11 12 13 14 15 16 17 18

Statistical interpretation of data.

An analysis of variance was carried out by Dr. Andrew Carothers at the Computer Section of the MRC Clinical and Population Cytogenetics Unit. This showed that (i) there were no significant differences between preparations within individuals, (ii) that the intra individual variance component for man, chimpanzee and orangutan did not differ significantly (pooled estimate 4949.3 with 485 d.f.), but were significantly greater in the gorilla (11606.7 with 197 d.f.). Confidence intervals were constructed using these estimates and taking values of the interindividual intra-species component of either 2000 A.U.$^2$ (assumption A) or 1000 A.U.$^2$ (assumption B). The assumptions correspond to situations in which one individual in a hundred can be expected to differ from its species mean by the equivalent of two (A) or one (B) medium sized chromosome respectively. The implied degree of aneuploidy suggests that these are overestimates, as are the widths of the corresponding confidence intervals (Table 10.1). This is in accordance with the generally accepted fact pointed out by Vendrely and Vendrely (1948) that DNA is constant between chromosomally normal members of the same species.

(E) Discussion.

A comparison between the mean haploid dry mass of the spermatozoa of man and the great apes (Table 10.1) has shown that man has the lowest DNA content of the Hominidae, whereas the gorilla has the highest. Mirsky and Ris (1951) have pointed out that phylogenetically related species have similar amounts of DNA, and a comparative study of the DNA content of diploid cells in man and

18/

TABLE 10.1

COMPARISONS OF TOTAL DRY MASS OF SPERMATOZOA OF THE GREAT APES AND MAN

| SPECIES | MEANS | DIFFERENCE | APPROXIMATE 95% CONFIDENCE LIMITS ASSUMPTION A LOWER | ASSUMPTION A UPPER | ASSUMPTION B LOWER | ASSUMPTION B UPPER |
|---|---|---|---|---|---|---|
| CHIMPANZEE - HUMAN | 810.0 - 742.3 | + 67.7 | + 21.8 | +113.6 | + 41.8 | + 93.6 |
| ORANGUTAN - HUMAN | 871.6 - 742.3 | +129.3 | + 73.0 | +185.6 | + 97.6 | +161.0 |
| GORILLA - HUMAN | 965.2 - 742.3 | +222.9 | +166.4 | +279.5 | +190.7 | +255.2 |
| ORANGUTAN - CHIMPANZEE | 871.6 - 810.0 | + 61.6 | + 5.3 | +117.9 | + 29.9 | + 93.3 |
| GORILLA - CHIMPANZEE | 965.2 - 810.0 | +155.2 | + 98.7 | +211.8 | +123.0 | +187.5 |
| GORILLA - ORANGUTAN | 965.2 - 871.6 | + 93.6 | + 28.5 | +158.9 | + 56.6 | +130.7 |

The component of variance between individuals within a species is taken to be either 2000 A.U.$^2$ (assumption A) or 1000 A.U.$^2$ (assumption B).

18 other species (Atkin et al, 1965) has shown that the DNA content of man is roughly similar to that in all placental mammals. Manfredi-Romanini (1972, 1973) compared the DNA content of lymphocytes of man and a large group of primates including three species of great apes. In these studies it was reported that man had a lower DNA content than any of the great apes, but the direction in which DNA was found to increase was man < gorilla <chimpanzee < orangutan. These results do not correspond to ours in which the order was man < chimpanzee < orangutan < gorilla. It must be pointed out that these studies were made using Feulgen stained lymphocyte cells which have well condensed nuclei in which cytophotometric factors have been found to affect measurements of DNA content with the Feulgen reaction (Bedi and Goldstein, 1974). However, the results reported by Manfredi-Romanini (1971, 1973) point to the fact that the great apes and man have conserved within close limits, the same amounts of DNA as the Lemuroidea (lemurs), the Cercopithecoidea (old world monkeys) and the Cebidae (new world monkeys). The Hylobatidae (gibbons) seem to be an exception in showing the lowest DNA content of all primates. It is tempting to speculate whether speciation has occurred with a concomitant increase or decrease of DNA content. Sparrow et al (1972) have shown that in nature, DNA content may range from 0.007 pg (1 pg = $10^{-12}$g) in bacteria to 100 pg in the haploid cells of salamanders and some plants. Ohno (1970, 1972) had pointed out that DNA increase must have occurred during evolution to allow a genome to acquire extra DNA capable of accumulating mutations free from selective pressure. A generally held view is that DNA has obviously increased in the evolution of invertebrates (Mirski and Ris, 1951), but the evolution of/

of land vertebrates has occurred with a decrease of DNA content. Hinergardner (1976) has reviewed the literature of DNA content in relation to evolution, and concludes that for a given taxon, the more generalized species (those showing more characters in common with other members of the taxon) have more DNA than those which are more specialized (those who have unique characters which are rare among other members of the same taxon). Specialization is then accompanied by a decrease in DNA, since each step of specialization is achieved by favourable selection of DNA sequences already present in the genome. At the same time, other DNA sequences are released from the pressure of selection, and are therefore allowed to degenerate or to disappear.

In relation to DNA content and chromosome number, there seems to be no correlation in mammals (Bachmann, 1972), and the fact that man has 46 chromosomes against 48 of the great apes as result of a telomeric fusion cannot explain the differences in DNA content observed between species. Manfredi-Romanini and Campana (1971) compared the DNA content of two populations of rats, one of which had suffered a Robertsonian fusion. No significant difference was detected between them as a result of the chromosome rearrangement. Though not identical, this kind of rearrangement is comparable to the one that took place in the evolutionary branch leading to man, after this line split from the common stock of the Hominidae. The DNA content of two phylogenetically related species with equal chromosome numbers and very similar banding patterns such as the ox and the goat (Evans et al, 1973) have been shown to have differences in their DNA content of approximately 15% (Sumner and Buckland, 1976). Since the amount of satellite DNA is approximately the same in these

two/

two species, it was postulated that the extra DNA of the ox must be evenly distributed throughout the genome. In the Hominidae, in which chromosome numbers are 48 in four species and 46 in one species, and 99% of the G- (or R-) chromosome bands are common to all species (Dutrillaux, 1975), this might also be the case. There is evidence that the great apes might have a larger amount of satellite DNA sequences than man (Gosden et al, 1977; Mitchell et al, 1977). It is improbable, however, that such differences in satellite DNAs between species could explain differences between total amounts of DNA, since in man, the amount of satellite DNA is very low, and probably represents no more than 6% of the diploid genome (Corneo et al, 1967, 1970, 1971, 1972). It is probable that in the great apes, as in man, satellite DNA may comprise only a small percentage of the total genome. Thus, the reason why man has less DNA than any of his closest living relatives is still obscure, and no simple straightforward explanation can be advanced at this time.

In relation to DNA content and morphology of spermatozoa, it is interesting that man showed no significantly different variability in haploid DNA content than the chimpanzee and the orangutan, although these latter two species do not have the degree of morphological pleomorphism in their spermatozoa characteristic of man. Thus it must not be assumed that morphologically abnormal spermatozoa are necessarily genetically defective, or that the number of aneuploid spermatozoa might be very frequent in man as it has been postulated (Geraedts and Pearson, 1976). The gorilla, like man, produces pleomorphic spermatozoa but also shows a greater variation in DNA content. The production of pleomorphic spermatozoa in the gorilla is obviously not related to clothing induced testicular hyperthermia, as has been suggested/

suggested for man (Bedford, 1974).

Finally, there is no explanation for the observation that the gorilla produces spermatozoa which are more variable in DNA content. The very high frequency of chromosome polymorphisms in this species (Q- and C-band polymorphisms) might be one of the factors contributing to the greater variability of DNA content in its spermatozoa. However, a complete explanation of this finding will await further investigation.

(F) Summary of this chapter.

(1) Man was found to have the lowest spermatozoal DNA content of the Hominidae, and the gorilla the highest. The implications of changes in the amount of DNA in speciation has been discussed, as well as the possible factors involved.

(2) Determinations of total dry mass of spermatozoa showed that the gorilla produces spermatozoa which are more variable in DNA content than all other species. Both the gorilla and man produce pleiomorphic spermatozoa, but in man, pleiomorphism is not positively correlated with a greater variance in haploid DNA content when compared to the chimpanzee and the orangutan.

(3) Similar proportions of diploid spermatozoa are produced in the gorilla and in man.

# SECTION III

# APPENDIX

## (A) PROCEDURES USED IN SECTION I (CHROMOSOME STUDIES).

### Collection of Material.

Blood samples were obtained from animals kept in captivity at Yerkes Primate Research Centre (Atlanta, USA), Bristol Zoo (England) and Regent's Park Zoo (London, England). Detailed information on the number of animals, their location and their pedigrees is given in Section I, Chapters 2, 3 and 4 of the thesis. Ten mls of venous blood were withdrawn with a heparinized syringe and transferred to a heparinized tube. The blood sample was mixed gently after collection to allow an adequate dilution of the heparin, and then the tubes were tightly sealed and shipped to Edinburgh.

### Blood culture

Following their receipt, the tubes were put in an Eschmann rotator for 30 mins. and the contents allowed to mix thoroughly. Whole blood was cultured by a modification of the blood culture microtechnique of Hungerford (1965). Culture medium was prepared as follows:

(1) A volume of Ham's F-10 culture medium (Gibco-Biocult Ltd., Glasgow) was supplemented with tryptose phosphate broth (10%) and fetal calf serum (10%) (Difco, Detroit, USA).

(2) The final volume was divided into aliquots of 10 mls which were put in 30 ml sterile glass Universal containers.

(3) 30 units of lithium heparin (Evans Medical Ltd., Liverpool) were added to each container by adding 0.03 ml of an aqueous solution of concentration 1000 u/ml.

(4) A vial of purified Phytohaemagglutinin (Wellcome Reagents, England) was dissolved in 5 mls of sterile distilled water. The vial contained/

2mg (18.8 mitogenic units). A volume of 0.6 mls of this solution was added to each container to give a concentration equivalent to 0.2256 mitogenic units per container.

(5) A volume of 0.6 mls of total blood was then added to each container. The cap was tightly closed and the final cell suspension was allowed to mix thoroughly. Incubation was carried out in a water bath at 37°C for 72 hours before harvesting.

(6) Three and a half hours before the completion of the incubation period 0.5 ml of a $10^{-5}$M solution of colchicine (McFarlane Robson Ltd., Glasgow) in sterile distilled water was added to each container.

Harvesting of cultures.

(1) The tubes were removed from the water bath and shaken vigorously to remove all cells from the surface of the container. The contents were emptied into a centrifuge tube.

(2) An initial centrifugation (2,000 rpm for 5 mins) was carried out, and the supernatant was discarded by carefulpouring or by aspiration with a Pasteur pipette. The remaining cell pellet was resuspended in a hypotonic solution of 6 mls of potassium chloride (0.0075 M) for 10 mins. at room temperature.

(3) The suspension was centrifuged again as previously mentioned, the supernatant discarded and the pellet gently resuspended in 1 ml of remaining hypotonic solution. Freshly prepared fixative (3:1 glacial methanolacetic acid) was gently poured and allowed to mix with the cell suspension. Six mls of fixative were used and the cells were allowed to fix at room temperature for 10 mins.

(4) The cell suspension was centrifuged again as previously mentioned and/

and the supernatant discarded. The remaining cell pellet was resuspended two more times in fixative and centrifuged.

(5) After the third centrifugation, the pellet was resuspended in 1 ml of fixative.

Preparations of slides.

Precleaned glass slides were used. They were washed in absolute alcohol (ehtanol) and polished with gauze. One or two drops of the final cell suspension were dropped onto the slides which were waved vigorously to enhance the drying. Preparations were checked for an adequate cell concentration, mitotic activity and quality of spreading by observation under a phase contrast microscope.

Chromosome banding techniques and stains.

Q-banding.

Q-banding, as described by Robinson and Buckton (1971) has been used.

(1) One or two week old slides were stained for 6 mins. in 0.5% (W/V) quinacrine dihydrochloride (Sigma Chem. Co. St. Louis, USA) in deionised water, at a pH 5.5 to 6.0 at room temperature.

(2) The slides were then washed under running tap water for 3 mins., and mounted in deionised water.

(3) The slides were blotted to remove excess water by gently pressing the coverslip with a filter paper, thus allowing a very thin layer of water between the slide and the coverslip.

(4) The edges of the slide were then sealed with a rubber solution.

Observations./

Observations.

Stained slides were always observed on the same day with a Leitz Ortholux microscope with HBO 200 W/4 mercury lamp and Ploem setting No.3, with a BG 12 exciting filter and a 510 nm barrier filter. A X 70 objective was used for observation and photography.

Photography.

Photographs were taken with a Leitz Orthomat camera using Panatomic X film (Kodak). The film was developed in D-75 developer (Kodak) for 8 mins. at 20°C. The reaction was stopped by removing the developer and pouring a stopper solution (Kodak) for 1 min. The film was later fixed with Kodafix (Kodak) for 5 mins., washed under running tap water for 15 mins., rinsed in distilled water for 1 min. and allowed to dry at room temperature. Prints were made with Veribrom paper No.2 and 3 (Kodak).

C-banding.

This technique was used on preparations which were previously Q-banded, and the procedure was that described by Sumner (1972) with minor modifications:

(1) The slides were initially washed for at least 6 hours under running tap water to remove quinacrine staining.
(2) Three consecutive baths in ethanol 50%, 70% and absolute alcohol were used after washing.
(3) The initial denaturation in 0.2 M hydrochloric acid as described by Sumner (1972) was omitted.
(4) A freshly prepared 5% (W/V) barium hydroxide in distilled water was/

was kept at 50°C in a water bath, and the slides were immersed in this solution for 30 seconds.

(5) The slides were then washed in distilled water and dried. They were incubated in a Coplin jar containing 50 mls of 2 X SSC (SSC = 0.3 sodium chloride containing 0.03 trisodium citrate) at 60°C for 1 hour.

(6) After incubation, the slides were washed in distilled water, dried and stained with Giemsa for 10 mins. The staining solution was prepared with 2 mls of Giemsa solution (Giemsa Gurr R-66, England) in 40 mls of deionised water.

Photography.

Cells were photographed with a Leitz Orthomat camera using Microfilm film (Kodak) and a X 100 objective. Only cells that were previously identified with quinacrine were photographed. The film was developed with DG-10 developer (Kodak) for 5 mins. at 20°C. The reaction was stopped with a stopper solution (Kodak) and the film fixed with Kodafix (Kodak) for 5 mins. It was then washed under running tap water for 15 mins., rinsed in distilled water and allowed to dry at room temperature. Prints were made with Veribrom paper No.2 and 3 (Kodak).

G-banding.

The procedure described by Gallimore and Richardson (1973) was used as follows:

(1) One week old slides were incubated in 2 X SCC for 1 hour at 60°C.

(2) The slides were then washed in deionised water and dried.

(3) The slides were then immersed in a 1% trypsin solution (Bacto-Trypsin/

Trypsin, Difco, Detroit, USA) in distilled water for 20 seconds.

(4) The slides were washed carefully with distilled water to remove the trypsin completely.

(5) Staining was with a Giemsa solution prepared as previously described for C-banded preparations for 8 to 10 mins.

(6) The slides were put in xylene for 24 hours and then mounted in XAM neutral medium (Gurr, England).

Photography.

Cells were photographed using a Leitz Orthomat camera with Panatomic-X film (Kodak) and a X 100 objective. The development of the film and the preparations of prints were identical to the procedure described for Q-banded preparations.

R-banding.

The procedure described by Sehested (1974) was used in the following way:

(1) Fresh slides, preferably no older than two days, were incubated in a 1 M solution of sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot {}_6H2O$) in distilled water at pH 4.5 - 5.5 at 88°C for 10 mins.

(2) The slides were gently agitated in the Coplin jar during incubation to avoid the formation of bubbles on the chromosomes.

(3) After incubation, the slides were rinsed in distilled water.

(4) Staining was with a Giemsa solution prepared as for C-banded preparations.

Photography/

Photography.

Photographs were taken with a Leitz Orthomat camera using Microfilm film (Kodak) and an X 100 objective. The film was developed with D-76 developer (Kodak) for 8 mins. at 20°C. The reaction was stopped with a stopper solution (Kodak) and the film was fixed with Kodafix (Kodak) for 5 mins. The film was washed under running tap water for 15 mins., rinsed in distilled water and allowed to dry at room temperature. Prints were made with Veribrom paper No.2 and 3 (Kodak).

Chromosome analysis.

At least 10 cells of the G-banded preparations were analysed per animal. In Pan troglodytes, Pan paniscus and Gorilla gorilla at least 10 cells were also analysed with Q- and C-banding. In Pongo pygmaeus where there is no brilliant fluorescence, no terminal heterochromatic regions and no secondary constrictions in any part of the chromosome complement, Q- and C- banding was only carried out on four animals. This was done mainly to identify chromosome 9 (the normal and the variant type), the Y chromosome and the deleted chromosomes 14 and 22. The criteria of chromosome nomenclature and homologies between species was that of the Paris Conference (1971); supplement (1975), although some modifications have been proposed. The following abbreviations from the Paris Conference were used to denote the chromosomes of the different species: HSA = Homo sapiens; PTR = Pan troglodytes; PPA = Pan paniscus; GGO = Gorilla gorilla; PPY = Pongo pygmaeus.

Procedures used to study the late replication pattern of the chromosomes of man and the great apes.

Two different protocols were used to study the pattern of late replication, one in which thymidine was incorporated in the last five hours of the cell cycle before harvesting (T-pulse), the other in which BUdr was used as a thymidine substitute during the last five hours of the cell cycle before harvesting (B-pulse). Blood cultures of two human controls (one male and one female), two chimpanzees (Pan troglodytes), one male and one female, two gorillas (Gorilla gorilla), one male and one female, and two orangutans (Pongo pygmaeus), one male and one female, were used.

Protocol for T-pulse cultures.

(1) A volume of tissue culture medium not containing thymidine, RPMI 1640 supplemented with 5% glutamine (Gibco Bio-cult, Glasgow) was supplemented with tryptose phosphate broth (10%) and fetal calf serum (10%)(Difco, Detroit, USA).

(2) The volume was divided into aliquots of 10 mls which were put in 30 ml sterile glass containers.

(3) Lithium heparin and purified Phytohaemagglutinin were added to each container as previously described for cell cultures in page

(4) 0.1 ml of a 1mM solution of BUdr (5-bromodeoxyuridine) in distilled sterile water was added to each container.

(5) 0.6 mls of total blood was added to each container. The cell suspension was mixed thoroughly and the tubes covered with dark tape or nylon to avoid light exposure.

(6) Incubation was at 37°C in a water bath for 48 hours.

(7) Five hours before the termination of the incubation period the containers/

containers were centrifuged at 2,000 rpm for 5 mins. and the supernatant discarded. The cell pellet was resuspended in 10 mls of RPMI 1640 with 5% glutamine (Gibco-Biocult, Glasgow). It was centrifuged again and the supernatant was discarded.

(8) The cell pellet was resuspended in 10 mls of tissue culture medium prepared as specified in points (1), (2) and (3).

(9) A volume of 0.1 ml of a 1 mM solution of thymidine was added to each container.

(10) Three and a half hours before the termination of the incubation period 0.5 ml of a $10^{-5}$ M solution of colchicine in distilled water was added to each tube.

(11) At the termination of the incubation period (48 hours) the cultures were harvested in the same way as explained in page 170.

Protocol for B-pulse cultures.

(1) A volume of tissue culture medium RPMI 1640 with 5% glutamine (Gibco-Biocult, Glasgow) was supplemented with phosphate tryptose broth (10%) and fetal calf serum (10%) (Difco, Detroit, USA).

(2) The volume was divided into aliquots of 10 mls which were put into 30 ml sterile glass containers.

(3) Lithium heparin (Evans Medical Ltd., Liverpool) and purified Phytohaemagglutinin (Wellcome Reagents, England) were added to each container as previously described in page 169.

(4) A volume of 0.1 ml of a 1mM solution of thymidine in distilled sterile water was added to each container.

(5) A volume of 0.6 ml of total blood was added to each container, the containers were tightly closed and the cell suspensions mixed thoroughly.

(6)/

(6) Incubation was in a water bath at 37°C for 72 hours.

(7) Five hours before the termination of the incubation period 0.1 ml of a 1mM solution of BUdr in sterile distilled water was added to each tube.

(8) Three and a half hours before the termination of the incubation period 0.5 ml of a $10^{-5}$M solution of colchicine in distilled water was added to each tube.

(9) The cultures were harvested at the end of the incubation period and chromosome preparations were obtained in the same way as described in page 170.

Staining technique.

Chromosome preparations, obtained with both protocols, were stained according to the technique of Perry and Wolff (1974).

(1) Slides were stained for 10 mins. at room temperature in a 1% (V/V) solution of Hoechst 33258 50 mg/litre solution in distilled water.

(2) After staining the slides were washed under running tap water for 2 mins.

(3) Slides were dried and mounted in distilled water, and the coverslips were sealed with a rubber solution.

(4) The slides were then allowed to stay on the laboratory bench for 24 hours exposed to natural light.

(5) After that period, the coverslips were removed and the slides were incubated in a 2 X SSC at 60°C for 30 mins.

(6) Slides were stained with Giemsa as previously described for C-banded preparations.

Photography./

Photography.

Cells were photographed and prints were obtained as previously described for G-banded preparations (page 174 ).

Chromosome analysis.

The chromosomes of the species were ordered following the standard criteria for man and the great apes (Paris Conference 1971: supplement (1975). The T-pulse cultures produce cells which resemble a G-banding pattern, whereas the B-pulse cultures produce cells that resemble an R-banding pattern. A minimum of ten cells were analysed per individual.

Procedures used in the study of chromosome banding patterns in the Hominidae in relation to the patterns of in situ hybridisation to human cRNA I, II, III and IV.

This project was undertaken as a conjoint investigation with other members of the MRC Clinical and Population Cytogenetics Unit. The basic procedures of the experiment were as follows:-

(1) Four satellite DNA fractions (I, II, III and IV) were isolated from human DNA (following the procedure described by Gosden et al (1975)).

(2) Chromosome preparations were obtained from three species of great ape (Pan troglodytes, Gorilla gorilla and Pongo pygmaeus). Blood cultures were used to obtain chromosome preparations of Gorilla and Pongo as described in page 169. Chromosome preparations from Pan troglodytes were obtained from fibroblasts following the technique reported by Mitchell et al (1977).

(3) Chromosome preparations were stained with quinacrine as described by Robinson and Buckton (1971), and observed with the fluorescence microscope. An average of 15 cells were photographed per slide; an average of 6 slides were photographed per species for each of the four experiments described in (4).

(4) Each of the four human satellite DNAs isolated in (1) was used as a template for the in vitro synthesis, by a bacterial DNA dependent RNA polymerase, of a radioactively complementary RNA (cRNA I, II, III and IV) which was applied to the previously quinacrine stained chromosome preparations after denaturation with HCl, as described/

described by Gosden *et al* (1975).

(5) Following autoradiography, cells that had previously been selected under fluorescence were relocated, and rephotographed. Chromosome identification was made on autoradiographed metaphase chromosomes identified by reference to the fluorescence photographs.

(6) The distribution of silver grains was analysed on photographic prints as described by Mitchell *et al* (1977). Each chromosome was divided into a number of equal sized segments, the segment size being approximately equal to half of the smallest chromosome (No.22 in man; No.23 in the apes). The number of grains in each segment were counted in twenty or more cells, and the average number was plotted in a histogram. Where an area was saturated with grains, a grid with the dimensions of an average grain diameter (1.2 $mm^2$) was used to estimate the minimum number of grains in the area. The average number of grains above background on each chromosome was calculated as a percentage of the average total number of grains per metaphase.

## (B) PROCEDURES USED IN SECTION II (STUDIES ON SPERMATOZOA).

### Material.

Semen samples were obtained from 4 human donors of proven fertility. Semen samples from 3 chimpanzees (Pan troglodytes), also of proven fertility, were supplied by Edinburgh Zoo. In all these cases, the ejaculate was obtained by masturbation. Semen samples from two chimpanzees (Pan troglodytes), one pygmy chimpanzee (Pan paniscus), two gorillas (Gorilla gorilla), and two orangutans (Pongo pygmaeus) were obtained by electroejaculation following the method described by Warner et al (1974). The animals were phenotypically normal and all of proven fertility, except for one male orangutan who had never been mated. Electroejaculation was carried out at the Yerkes Regional Primate Center, Atlanta, USA.

### Preparation of the material.

(1) A volume of 1 ml of semen was put in a centrifuge tube and resuspended with a pipette in 6 mls of saline (0.9% sodium chloride). The suspension was centrifuged at 2,000 rpm for 5 mins. and the supernatant discarded. This operation was repeated twice, to remove seminal fluid from the spermatozoa.

(2) After the third centrifugation, the cell pellet was resuspended in 1 ml of saline, and 6 mls of freshly prepared fixative (3:1 methanolacetic acid glacial) was added carefully to avoid the formation of cell clumps. The material was allowed to fix for 10 mins. at room temperature.

(3) The final suspension was centrifuged, and the cell pellet resuspended in 6 mls of fixative and centrifuged again. This operation/

operation was repeated until a total of three consecutive washings in fixative and subsequent centrifugation were completed.

(4) The final pellet was resuspended in a volume of 1 ml fixative and air dried preparations were obtained. Semen samples obtained at Yerkes Research Primate Center were processed in that laboratory and semen samples given by the Edinburgh Zoo were processed by the candidate.

Morphological examination of spermatozoa.

A staining technique, derived from that reported by Papanicolaou (1949), was used:-

The slides were:

(1) rehydrated by washing them successively for 1 minute in (a) absolute ethanol, (b) 70% ethanol, (c) 50% ethanol, and distilled water;

(2) stained with Harris Haematoxylin (BDH Chemical Ltd., Poole, England) for 4 minutes;

(3) washed in running tap water until water became clear of stain coming out of the preparations;

(4) dipped in 0.5% (V/V) hydrochloric acid in water for 2 seconds;

(5) washed in distilled water for 5 minutes;

(6) dipped in lithium carbonate 1% (W/V) in water for one minute;

(7) washed in distilled water;

(8) passed through three successive baths of (a) ethanol 50%, (b) ethanol 70%, and (c) absolute ethanol;

(9) stained in Orange "G" (BDH Chemicals Ltd., Poole, England) for 5 minutes;

(10)/

(10) washed in ethanol 50% 1 minute;

(11) stained in EA 50 (BDH Chemicals Ltd., Poole, England);

(12) washed in ethanol 50% for 1 minute and in absolute ethanol for 1 minute;

(13) dipped in xylene and mounted in HAM neutral medium (Gurr, England).

200 spermatozoa per individual were scored. In Papanicolaou stained preparations the criteria used in classifying spermatozoa according to their morphology was as follows:

(1) In the two species of chimpanzees and in the orangutan the spermatozoa were of a very uniform type with few exceptions. The very regular type of spermatozoa was the one considered "normal".

(2) In man and the gorilla, in which a high number of pleomorphic spermatozoa were found, the word "normal" refers to the "modal" type of cell found in the ejaculate.

Additional morphological observations of spermatozoa were made with a Nomarski interference microscope. Photographs were taken of unmounted clean preparations with a Leitz Orthomat camera and an X 100 objective. The film used was Panatomic X (Kodak) and the film was developed as described in page 174 for G-banded preparations. Prints were obtained with Veribrom paper No.4 (Kodak).

Procedure used for the detection of fluorescent ("F") bodies.

Staining with quinacrine was identical to the procedure described by Robinson and Buckton (1971) for chromosome preparation and it has already been described in page 171 of the Appendix.

Photography and printing were carried out in the same way as described/

described for quinacrine stained chromosomes.

Technical considerations.

The "F" bodies were more clearly visible at the region of the acrosomic cap or in the boundary between that region and the more densely stained region of the head. Visualising "F" bodies in spermatozoa down the microscope was obviously easier than photographing them, especially when more than two "F" bodies were detected. Since the "F" bodies were located at different planes of the head, correct visualization could be achieved by focussing up and down on the microscope, but it was impossible to photograph more than two "F" bodies in the same cell.

Dry mass estimations.

Dry mass estimations were made on unstained preparations which were washed under running tap water before measuring. Only very clean preparations with well-spread, non-overlapping spermatozoa were used in order to avoid a false estimation of the optical path difference of the background. The principles and procedures used in dry mass estimations are discussed in Section II, Chapter 10 , and will not be repeated in this section, except for details on how the Feulgen reaction was performed.

Feulgen staining was used to corroborate whether there was residual DNA after trichloroacetic extraction.

(1) The slides were hydrolysed at room temperature for 1½ hours in 5 M hydrochloric acid.

(2) They were washed in distilled water after hydrolysis.

(3)/

(3) Stained with Schiff reagent for ½ an hour at room temperature.

Preparation of the Schiff reagent followed the procedure reported by Lillie (1965):-

(1) A volume of 85 ml of boiling water was poured on to a basic fuchsin, 1.9 g. of potassium methabisulphite (anhydrous) and 15 ml of a 1N solution of hydrochloric acid.

(2) The mixture was shaken at intervals during two hours after which it became brownish or straw-coloured.

(3) Fresh decolourizing charcoal was added to the mixture (0.5 g) and it was shaken for a few minutes.

(4) The mixture was filtered, the filtrate being clear and colourless.

(5) The filtrate was stored in the refrigerator at $4^{\circ}C$, covered with aluminium paper to avoid light exposure. The solution was stored for a day before being used.

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# The chromosomal distribution of human satellite III DNA during meiosis

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## Abstract

In human meiotic cells DNA satellite III is located in the same sites as in mitotic cells, but in prophase the areas are less condensed. This differs from the situation in *Plethodon* where the sites of heavy satellite DNA are condensed throughout meiotic prophase (MacGregor and Kezer, 1971). The difference may be ascribed to the fact that *Plethodon* heavy satellite is pericentrically located, whereas few if any of the human satellite sites are actually at centromeres. A second difference is found in sperm, where the *Plethodon* satellite is located at a single site in the rear of the nucleus, while the satellite regions in man do not have a common or constant orientation, suggesting that the respective satellites may well be functionally different .

## Introduction

RNA complementary to human DNA satellite III (cRNAIII) hydridizes *in situ* mainly with chromosomes 9, 15 and the Y, and to a lesser extent with chromosomes 13, 14, 20, 21 and 22 of the human complement (Gosden *et al.*, 1975). A quantitative analysis of these results (op. cit.) has shown that 20% of total hybridization occurs at the secondary constriction of each chromosome 9; 20% at the heterochromatic segment of the Y chromosome; and 7% at the centromere/short arm region of each chromosome 15. Thus, these five chromosomes account for about 75% of the hybridization of cRNAIII to mitotic metaphase chromosomes, and this differentiates them from the rest of the karyotype while the different morphology of these chromosomes makes them easy to distinguish.

We now report on the *in situ* hybridization of c RNAIII in human meiotic cells in a study of DNA satellite III during meiosis and spermatogenesis.

## Materials and methods

Material was obtained from testicular biopsies of phenotypically normal, 46,XY fertile males undergoing vasectomy. Slides of meiotic chromosomes were prepared by the method of Evans *et al.* (1964). Satellite III DNA and cRNAIII were prepared, and *in situ* hybridization performed, as described previously (Gosden *et al.*, 1975).

## Results and discussion

Hybridization took place in interphase nuclei, chromosomes of dividing gonial cells and in nuclei of cells at all stages of the meiotic cycle including mature spermatozoa. Two C group, two D group and a G group chromosome could be clearly distinguished in spermatogonial metaphases. Dense blocks of grains were visible in the subcentromeric regions of two submetacentric chromosomes (chromosome 9) and on the long arm of a small chromosome of the G group (the Y). In many cells a block of grains could also be seen on the short arm of a pair of large acrocentric chromosomes. By analogy with

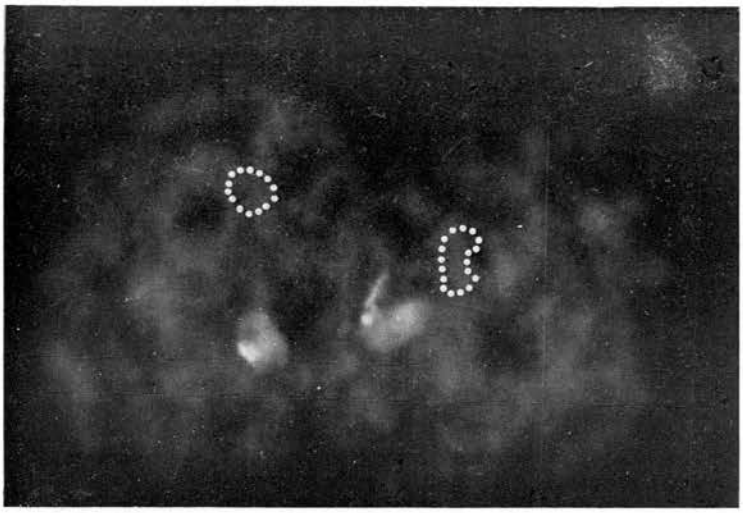

**Figure 1** Two cells in meiotic prophase (pachytene) stained with quinacrine and photographed under UV light. The sex vesicles are prominent in both cells, and each sex vesicle contains a brilliantly fluorescent area, the F body. The non-fluorescent are outlined in each cell is a major site of hybridization of cRNAIII (see Figure 2) x 2,000.

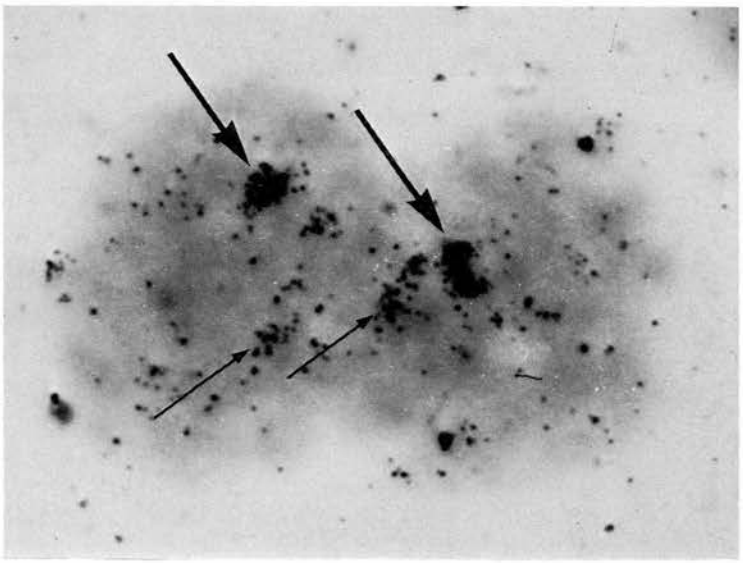

**Figure 2** Giemsa stained autoradiograph of the same cells after hybridization with cRNAIII. Short arrows indicate hybridization of the F bodies, which are the fluorescent regions of the long arm of the Y chromosomes. Long arrows indicate areas of very heavy labelling (outlined in Figure 1) which presumably contain the paired heterochromatic secondary constrictions of chromosome 9 (see text). x 2,000.

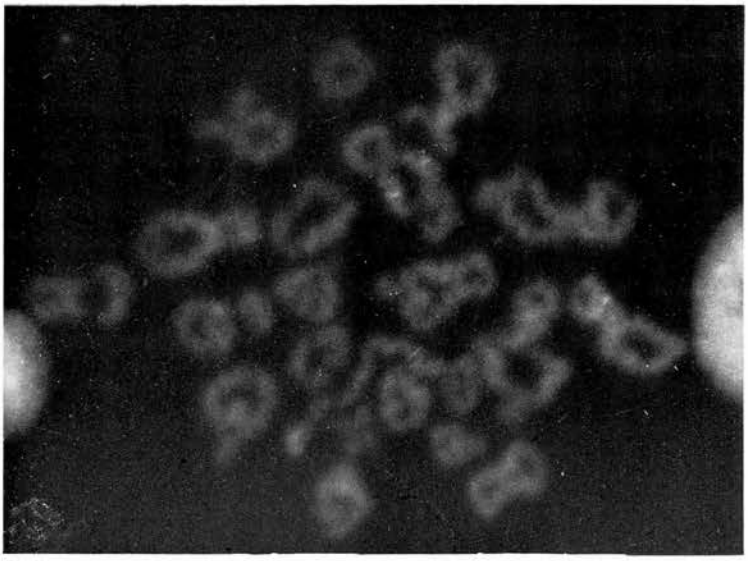

**Figure 3** Fluorescence photograph of quinacrine stained cell at diakinesis showing 23 elements: 22 bivalents and the XY complex. A number of chromosomes can be clearly identified by their morphology, size and fluorescence pattern, and these are indicated in Figure 4. Identification of the others can only be tentative, though the majority can be assigned to their size group. x 2,000.

the results obtained using blood lymphocyte chromosomes, these are assumed to be chromosome 15. A number of other sites of hybridization were seen, but the chromosomes concerned were less easily identified than in lymphocyte preparations.

Labelling was present in the early stages of meiotic prophase but the labelled areas were rather more diffuse than in mitotic interphase or prophase. However, in every cell there were two clearly distinct areas of high grain density (Figures 1 and 2). One of these is located in the fluorescent body (F body) identified by quinacrine staining prior to hybridization. The only area of bright fluorescence to which cRNAIII hybridizes in metaphase chromosomes is the distal part of the long arm of the Y chromosome, and this result is further confirmation of the identity between the F body and this chromosome region. The other densely labelled area must include the paired subcentromeric regions of chromosome 9, although other chromosomes which contain satellite III may be associated with this pair.

A number of chromosomes are seen to be clearly labelled at diakinesis (Figures 3 and 4). Among these, the extended heterochromatic regions of the paired chromosomes 9, the Y chromosome and several pairs of acrocentric chromosomes are distinct. It is not possible to identify these with certainty, but it is not unreasonable to assume that the relative amounts of hybridization seen in mitotic metaphase should be found in meiotic cells, and on this basis it is possible to assign tentative identities to these chromosomes. However, as in leptotene and pachytene, these sites of hybridization appear to be less condensed than in mitotic prophase. This observation differs from that of MacGregor and Kezer (1971) who found compact sites of labelling with heavy satellite cRNA in *Plethodon* on all centromeres throughout meiotic prophase. These results are not strictly

comparable as the satellite DNA in *Plethodon* is located in the immediate vicinity of, if not directly at, the centromere, while although some of the sites of hybridization in human chromosomes are close to centromere, in no case do they appear to be directly at the centromere, and the major sites on chromosome 9 and the Y chromosome are clearly separate from it. Extension of some of the secondary constrictions of human chromosomes (particularly chromosome 9) at meiotic metaphase has been observed previously (Hultén and Lindsten, 1970) and these results suggest that in man some of the heterochromatic regions are less condensed during meiosis than during mitosis, having undergone partial decondensation in the very early stages.

Secondary spermatocytes also show heavy labelling over chromosome 9. This chromosome can be identified in well-spread metaphases and shows heavy labelling at the secondary constriction on both chromatids. An acrocentric D chromosome labelled on both short arms may be identifiable as chromosome 15. In round spermatids and mature spermatozoa, hybridization occurs to a reduced level compared with the total hybridization in a meiotic cell. This can be partly accounted for by the reduction in DNA content (25% of that at diakinesis) but also because the extreme condensation of the chromatin must restrict access of the cRNA to its complementary DNA (Sumner, Mitchell and Gosden, in preparation). Some spermatids show two areas of dense labelling, while others show only one (Figure 5). These are probably the 9 and Y chromosomes in the first case (Y-bearing spermatid) and chromosome 9 in the second

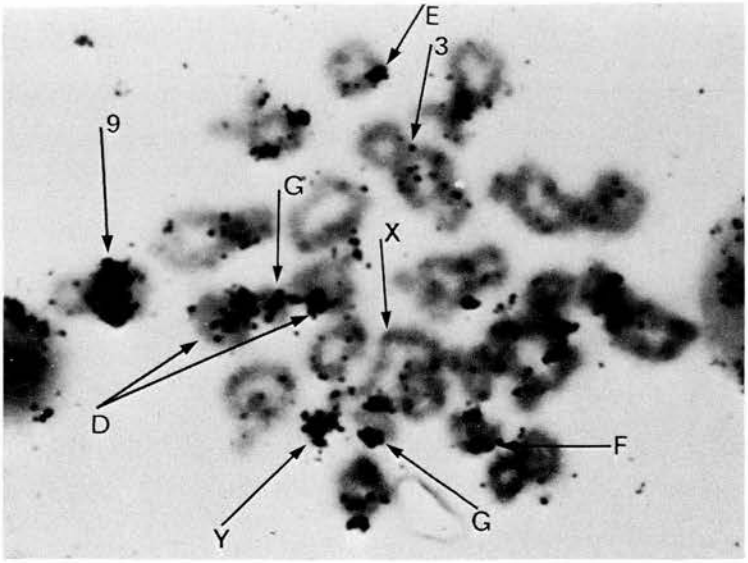


**Figure 4** Giemsa stained autoradiograph of the cell shown in Figure 3 after hybridization with cRNAIII. Note the very heavy labelling of the secondary constriction of the 9 bivalent. Other clearly labelled areas are the Y chromosome, two D group bivalents (presumably 14 and 15) and three bivalents of the F—G groups (presumably 20, 21 and 22). Note that the centromeres of one of these (at bottom, right) are labelled unequally, indicating that this individual is heteromorphic at this site (Gosden *et al.*, 1975) x 2,000.

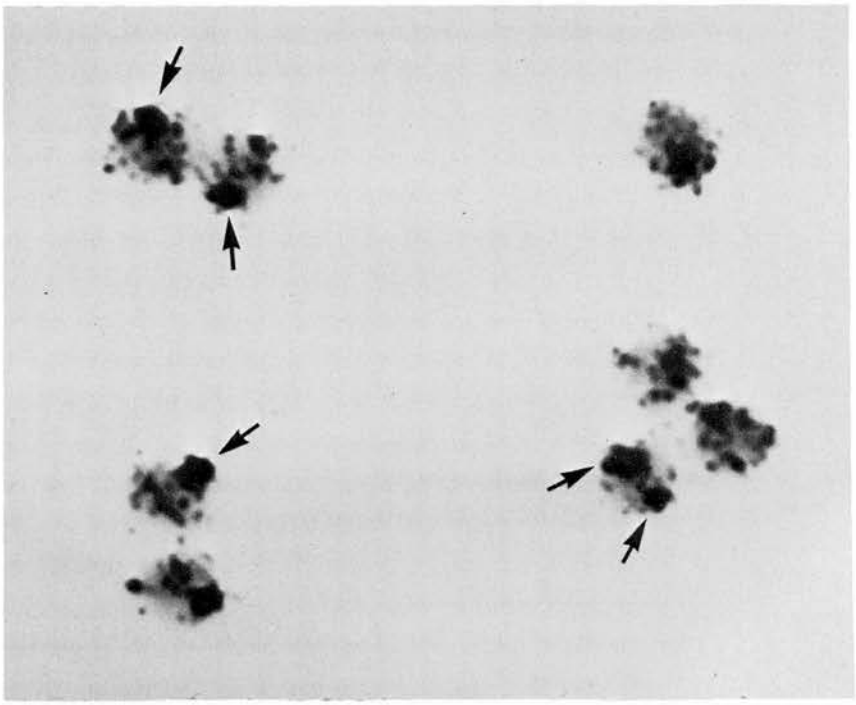

**Figure 5** Giemsa stained autoradiograph of round spermatids hybridized with cRNAIII. Arrows indicate spermatids with one or two sites of hybridization. x 2,000.

case (X-bearing spermatid). It was not possible to distinguish separate sites of labelling in the sperm head, since the area is much reduced and, if more than one site is labelled, the sites coalesce.

The location at which cRNAIII hybridizes in the sperm head varies. There is thus no evidence from this hybridization to suggest that there is a constant alignment of the condensed chromatin during spermatogenesis. These findings differ from those of MacGregor and Walker (1973) who found that *Plethodon* heavy satellite DNA hybridized to a single site in sperm nuclei located in the rear quarter of the nucleus. In *Plethodon*, satellite DNA is found in the centromeres in sperm. Chromosomes have been shown to have specific locations in the sperm of many organisms, but in man the F body has not been found at a constant location in the sperm. This, together with the results from the hybridization presented here indicates that the chromosomes in human sperm do not have a common or constant orientation and suggests that the satellites may differ functionally from the *Plethodon* satellite.

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# A chromosome rearrangement in an orangutan studied with Q-, C-, and G-banding techniques

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*Abstract*

A constitutional chromosome rearrangement in a phenotypically normal male orangutan is described. The rearrangement resulted from three breakages in chromosome 9 and involved a pericentric inversion and additional reciprocal transpositions of the terminal segments of both arms. Q-, G-, and C-banding studies were carried out, and it was shown that the chromosome affected was a member of the only pair in the complement that lacked a centromeric C-band and that its G-banding pattern closely resembled that of chromosome 12 in man. The origin of the rearrangement and the role of such rearrangements in producing chromosome polymorphisms are discussed, particularly in relation to evolution and speciation.

An additional pair of heteromorphic chromosomes (No. 23) is also described. The short arm satellited region of one member of the pair was found to be variable and strongly C-banded.

The chromosomes of the orangutan *(Pongo pygmaeus)* were originally described by CHIARELLI (1961), CHU and BENDER (1962), and BENDER and CHU (1963), and the karyotype was later compared with that of other hominoids (CHIARELLI, 1962; HAMERTON et al., 1963; KLINGER et al., 1963; EGOZCUE, 1969). The development of chromosome-banding techniques a decade later stimulated a number of workers to make detailed comparisons of banding patterns between human and hominoid primate chromosomes (PEARSON et al., 1971; DE GROUCHY et al., 1972; TURLEAU et al., 1972; BOBROW and MADAN, 1973; DUTRILLAUX et al., 1973; EGOZCUE et al., 1973*a, b*; DE GROUCHY et al., 1973; LEJEUNE et al., 1973;

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LIN et al., 1973; PEARSON, 1973; TURLEAU and DE GROUCHY, 1973; WARBURTON et al., 1973; MILLER et al., 1974; DUTRILLAUX et *al.*, 1975*a, b*), and these studies have demonstrated considerable homologies between chromosomes and chromosome arms in man, gorilla *(Gorilla gorilla),* chimpanzee *(Pan troglodytes),* and orangutan. Most of this work laid emphasis on homologies with the human chromosome complement, and few of these studies have provided information on banding patterns in the orangutan. TURLEAU et al. (1972) and later DE GROUCHY et al. (1973), using R-banding techniques, showed considerable homologies between certain orang and human chromosomes, and PEARSON (1973), using Q-banding, reported similar findings. DUTRILLAUX et al. (1975*b*) have reported a comparative study of the chromosomes of the orangutan with those of man and hominoid apes using Q-, R-, T-, and H-bands. In the Q-banding studies, it was further shown that, in contrast to man, gorilla, and chimpanzee, none of the orang chromosomes are characterized by having regions of brillant fluorescence—a finding taken to be in line with the generally held view (see SOKAL and SNEATH, 1963) that the orangutan had evolved from the ancestral stock before the emergence of the lineage from which the African apes and man originated.

We have recently had blood samples from two male orangutans and have studied their chromosomes using Q-, G-, and C-banding techniques (PARIS CONFERENCE, 1971). Our studies show that one animal was heterozygous for a large structural rearrangement (inverted insertion) in one chromosome that lacks a centromeric C-band (chromosome pair 9) and showed polymorphism on chromosome pair 23.

## *Materials and methods*

Blood samples from two male (Sumatran) orangutans were supplied by the Yerkes Regional Primate Research Center (Emory University, Atlanta, Ga.). Both were normal, healthy animals of 2 years of age. Ten milliliters of venous blood was withdrawn in a heparinized syringe and later transferred to a heparinized tube. The tubes were sealed and shipped by air to Edinburgh. Following their receipt, they were put in an Eschmann rotator for 30 min, and their contents were allowed to mix thoroughly. Chromosome preparations were obtained by the standard technique of HUNGERFORD (1965).

Q-banding was by the method of ROBINSON and BUCKTON (1971). Slides stained with quinacrine were later washed and stained for the C-banding technique.

C-banding was done by the method of SUMNER (1972), except that the initial incubation in 0.2 M HCl was omitted and exposure to $Ba(OH)_2$ was reduced to

30 sec. Preparations that had not been previously fluoresced were also C-banded and compared with the C-banding following Q-fluorescence. This procedure was done for slides from both animals.

G-banding was done on separate slides, following the method of GALLIMORE and RICHARDSON (1973), and the G-banding pattern of the orangutan was compared with the human banding pattern following the criteria of the PARIS CONFERENCE (1971), SUPPLEMENT (1975). When Q-banding is described, the criteria of the degree of intensity of fluorescence is also that of the PARIS CONFERENCE (1971). For each technique 15 cells were analyzed in detail from each animal.

## *Results*

The orangutan has a diploid number of 48 chromosomes. Figure 1 shows a G-banded karyotype of one animal (named Santing), in which chromosomes have been grouped according to the standard criteria (PARIS CONFERENCE [1971], SUPPLEMENT [1975]). Of the autosomes, 13 pairs are metacentric and 10 acrocentric. The sex chromosomes are clearly differentiated from the autosomes by G-banding: The X, a submetacentric, is slightly longer than chromosome 6, although its centromere is more central and its banding pattern closely resembles that of the human X chromosome. The Y, a submetacentric, is longer and clearly different from chromosomes 22 and 23, the smallest acrocentrics.

The karyotype in fig. 1 shows that two pairs of autosomes, 9 and 23, are heteromorphic. The presumptively normal 9 (which was found in the other orangutan in both members of the pair), is a subtelocentric chromosome whose G-banding pattern closely resembles that of the human chromosome 12 (figs. 1 and 2). Its homolog is a submetacentric chromosome with a different banding pattern (figs. 1 and 2) which cannot be simply derived from the normal chromosome 9.

Figure 2 shows the detailed G-banding pattern of chromosome 9 and the suggested mode of origin of the rearrangement. The rearrangement can be defined as an *insertion* since it involves three breaks (PARIS CONFERENCE, 1971). The inverted region contains the centromere (pericentric inversion), and the rearranged chromosome can be symbolized as inv(9)(p13q13), with reciprocal direct transpositions (insertions) of the terminal segments of both arms, thus (reciprocal) ins(9)(p13q21q21p13).

Q-banding showed a similar pattern to G-banding for both members of the heteromorphic pair. C-banding showed that pair 9 (including the rearranged chromosome) is unique in the complement since it has no evident C-band. This apparent lack of C-banding was confirmed in cells

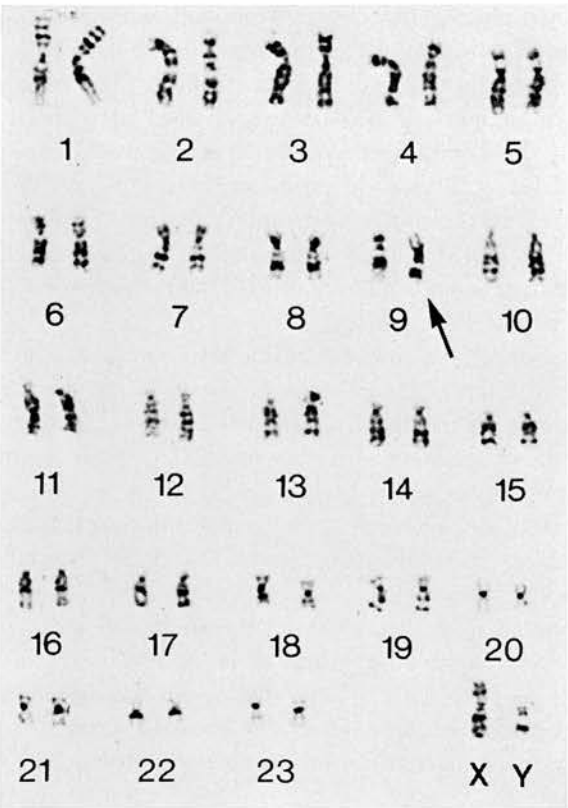


*Fig. 1.* The karyotype of the orangutan *(Pongo pygmaeus)* with G-banding. The arrow points at one of the two heteromorphic pairs of the complement. Pair 23 is also heteromorphic.

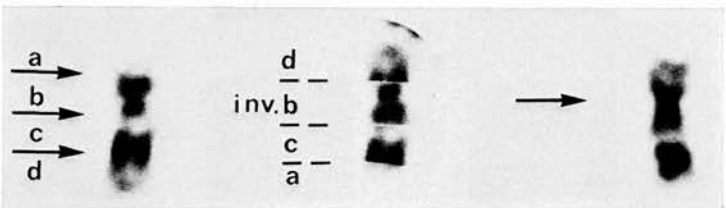


*Fig. 2.* Enlarged G-banded chromosomes (pair 9) showing the three suggested breakage points (arrowed). The letters *a, b, c,* and *d* represent the four unequal segments of the normal chromosome which, if rearranged (center), resemble the pattern of the abnormal chromosome (right).

from the same animal that were C-banded without being previously Q-banded and in cells from the second orangutan.

A second type of chromosome polymorphism was observed in pair 23 (fig. 3). This pair and pair 22 are the smallest acrocentrics and are of approximately the same length. With G-banding, both pairs can be easily differentiated since 22 has a prominent band below the centromere and 23 appears stained at the centromere and has a pale long arm. The two members of pair 23 differ in length due to the existence of a short arm in one of the chromosomes, making it submetacentric. This short arm appears pale with G-banding (fig. 1).

Pairs 22 and 23 can also be easily differentiated with fluorescence since 22 shows intense fluorescence below its centromere and becomes pale at the extreme at its long arm, whereas 23 shows a pale fluorescence all over the chromosome and is uniformly stained (fig. 3). It can be appreciated that polymorphism occurring in pair 23 accounts for the existence of a short arm in one of the members of the pair. With quinacrine, that short arm is pale, as is the rest of the chromosome. With C-banding, the short arm of the polymorphic 23 appears intensely stained. Chromosome associations among acrocentrics occur in the orangutan in a similar way to the satellite associations among human acrocentrics. In this case, where a member of pair 23 with a long short arm was found, and the area involved in chromosome association was enlarged, there was no difference in the frequency of association involving this chromosome as compared to its homolog.

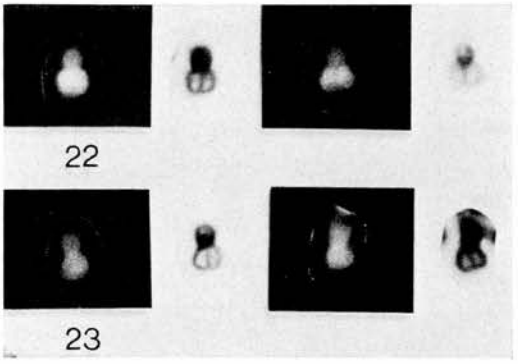


*Fig. 3.* Q- and C-banding patterns of the smallest acrocentrics, pairs 22 and 23 (from the same cell). Pair 23 appears paler with fluorescence than 22 and is clearly heteromorphic. Note the variable region of chromosome 23.

## *Discussion*

Our findings show that one of two specimens of orangutan was heterozygous for a complex rearrangement of chromosome 9, the rearrangement involving a pericentric inversion and a reciprocal transposition of the terminal segments of both arms. With G-banding, we have identified this chromosome as the homolog to chromosome 12 in man and with C-banding as the only pair in the complement that lacks a C-band at the centromeric region.

Chromosome anomalies, numerical and structural, have been previously reported in the Pongidae (MCCLURE et al., 1969; TURLEAU et al., 1972), sometimes leading to unfavorable conditions to carriers, similar to well-known clinical entities in man (MCCLURE et al., 1969). DUTRILLAUX et al. (1975) have reported a 48,XX chromosome constitution in a phenotypically normal male orangutan that also carried a heteromorphic pair as a consequence of a complex rearrangement affecting one chromosome which closely resembled No. 12 in man. This rearrangement, however, is not the same as that reported here. TURLEAU et al. (1975) have recently reported two different types of rearrangement in *Pongo pygmaeus*. One was a pericentric inversion in their chromosome 3 (our chromosome 2), and the other was described as an insertion of the centromere in their chromosome 12 (our chromosome 9). Both rearrangements were present in one adult male specimen of Bornean origin, and both were transmitted to two offspring.

It is probable that chromosome anomalies in the Pongidae may be common, and some of them might be responsible for deleterious conditions as in man. However, the animal we report in this paper is a phenotypically normal two-year-old, 48,XY male orangutan whose development and health has been satisfactory. Unfortunately, its young age did not permit us to assess its fertility. Since this animal is a heterozygote carrier for a complex rearrangement which includes a pericentric inversion, it is possible that this condition might affect its fertility, due to crossing over within the inversion loop at meiotic prophase. However, it need not exclude the production of balanced germ cells at meiosis, as in the case reported by TURLEAU et al. (1975), in which the carrier of two unrelated rearrangements had proven fertility.

If chromosome rearrangements which do not produce deleterious effects in carriers were transmitted to the offspring and spread in the population, speciation might well follow the model proposed by WHITE

(1968) in the absence of geographical isolation, as previously proposed by MAYR (1963). However, in the case of *Pongo pygmaeus,* in which the geographical distribution covers the islands of Sumatra and Borneo, it is remarkable that the rearrangement we describe in one specimen from Sumatra closely resembles the one recently found by TURLEAU et al. (1975) in the same chromosome in a specimen of Bornean origin. Although they are not necessarily identical rearrangements, it is unlikely that they reflect a consistent difference between Sumatran and Bornean subspecies of *Pongo pygmaeus,* but their finding might imply a relatively increased incidence of rearrangements involving chromosome 9.

Chromosome polymorphisms are not uncommon in the Pongidae, but most polymorphic regions reported are either very brilliantly fluorescent or intensely C-banded, suggesting that variation occurs in the amount of constitutive heterochromatin in some chromosomes. In the chimpanzee, chromosome polymorphisms involving the short arm regions of satellited acrocentrics have been reported by LIN et al. (1973) and by WARBURTON et al. (1973). In the gorilla, PEARSON (1973) has reported a polymorphic Q-band adjacent to the centromere of a large metacentric chromosome (No. 3); and DUTRILLAUX et al. (1973) reported variations in the short arm regions of those acrocentric chromosomes of the gorilla that are homologous to Nos. 13, 21, and 22 in man. More recently, MILLER et al. (1974) found that 12 autosomal pairs in *Gorilla gorilla* were variable in their Q- and C-banding patterns. Our findings in the orangutan show that the small pair of acrocentric chromosomes (No. 23), which strongly resemble No. 22 in man, may be heteromorphic. The polymorphic region stains intensely with Giemsa after alkali treatment, but, contrary to what is usually found for this region in the African apes and man, it does not fluoresce brilliantly and is as pale at the rest of the chromosomes.

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# A polymorphic structural rearrangement in the chromosomes of two populations of orangutan

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*Abstract*

A rearranged chromosome 9 was found in 12 of 23 specimens of orangutan, 4 of Bornean and 8 of Sumatran origin. Nine animals were heterozygous, and 3 were homozygous carriers for the variant chromosome, which was also traced in 4 other animals not studied by us. This type of chromosome rearrangement has been previously described (SEUÁNEZ et al., 1976) and is probably the same chromosome shown by LUCAS et al. (1973) and reported by TURLEAU et al. (1975) in other specimens. There is obviously a very high incidence of this variant chromosome 9 in *Pongo pygmaeus,* and it is unlikely that it could result from independent rearrangements occurring in unrelated specimens from two geographically isolated populations (Sumatran and Bornean). It is concluded that the rearrangement is of ancient origin and that it has been maintained in the populations of *Pongo* as a balanced polymorphism. This type of complex rearrangement resulting from two pericentric inversions, one inside the other, is compared with certain sporadic pericentric inversions in the human complement, with pericentric inversions which are polymorphic in other mammals, and with pericentric inversions involved in chromosome evolution in the Hominoidea.

The chromosomes of the orangutan *(Pongo pygmaeus)* have been analyzed in detail with chromosome-banding techniques, and their banding pattern has been compared with that of the human karyotype (PEARSON et al., 1971; TURLEAU et al., 1972; DE GROUCHY et al., 1973; LUCAS et al., 1973; PEARSON, 1973; DUTRILLAUX et al., 1975; TURLEAU et al., 1975; PARIS CONFERENCE, 1971, SUPPLEMENT, 1975). Wild populations of the orangutan exist on the islands of Sumatra and Borneo, and

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in a previous publication (SEUÁNEZ et al., 1976) we have described a complex chromosome rearrangement in one member of chromosome pair 9 in a normal male specimen of Sumatran origin. In this paper we report on the presence of this rearranged chromosome in a larger group of animals of both Sumatran and Bornean origin, and we compare our results with previous reports in the literature on the chromosomes of *Pongo pygmaeus*.

## *Materials and methods*

Blood samples from 23 animals were supplied by the Bristol Zoo and by the Yerkes Regional Primate Center, Atlanta, Georgia. Chromosome preparations were obtained by the standard technique of HUNGERFORD (1965).

Q-banding was by the method of ROBINSON and BUCKTON (1971). Slides stained with quinacrine were later washed and stained for the C-banding technique. C-banding was done by the method of SUMNER (1972) as modified for previously Q-stained slides (SEUÁNEZ et al., 1976). G-banding was by the method of GALLIMORE and RICHARDSON (1973) and R-banding by the method of SEHESTED (1974). The criteria of nomenclature and presumptive homology between the chromosomes of the orangutan and man was that of the PARIS CONFERENCE (1971), SUPPLEMENT (1975).

## *Results*

### *Data on the animals*

All animals were phenotypically normal and healthy and, in terms of origin, may be considered to fall into three groups: Bornean (B), Sumatran (S), and hybrids between individuals of the two populations (H). The origins and names of the animals are listed in the Appendix. All animals of the adult generation (B.I and S.I) were wild caught in Borneo or Sumatra, and all of the young generation (B.II, S.II, and H.I) were born in captivity. All adult animals had a proven fertility except for B.I.3 and B.I.4, which were recently mated for the first time, and S.I.16, which has never shown reproductive behavior, having never mated. However, S.I.16 as well as S.I.17 had been electro-ejaculated, and sperm morphology was found to be normal in both animals (see MARTIN et al., 1975); the production of regular-sized and shaped spermatozoa was above 98 % (SEUÁNEZ, unpublished). Two hybrid crosses involved Yerkes animals: one of a Sumatran father (S.I.17) and a Bornean mother (B.I.6), the other of a Bornean father (B.I.7) and a Sumatran mother (S.I.18). Both crosses resulted in progeny (fig. 1). This present report

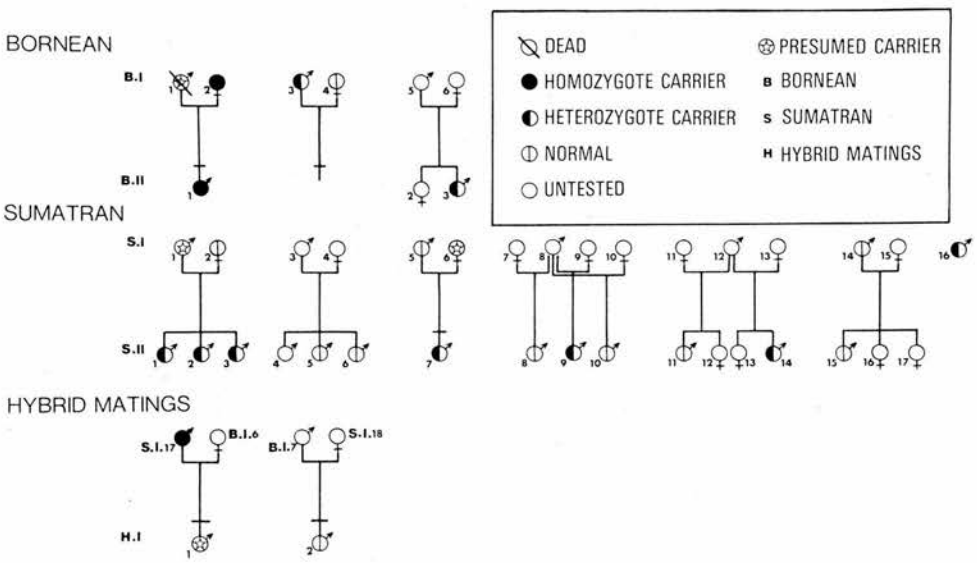


*Fig. 1.* Pedigree of the animals.

includes two animals previously studied by us (S.II.2 and S.II.5) (see SEUÁNEZ et al., 1976).

*Chromosome analysis*

Of the 23 animals, 9 were heterozygous for a rearranged chromosome 9, and 3 were homozygous carriers. The rearranged chromosome 9 was also traced in four other animals (fig. 1). The first, B.I.1, was the father of a homozygous carrier (B.II.1). The second, S.I.1, had been mated to a normal female (S.I.2) and was the father of three heterozygous carriers (S.II.1, S.II.2, and S.II.3). The third, S.I.6, had been mated to a normal male (S.I.5) and was the mother of a heterozygous carrier (S.II.7). Finally, the hybrid H.I.1 was the son of a homozygous carrier (S.I.17). In the other pedigrees, at least one animal in each of the three pairs B.I.5 and B.I.6, S.I.8 and S.I.9, and S.I.12 and S.I.13 are presumed to be carriers of this variant chromosome, as it has appeared in their offspring.

The variant chromosomes 9 were analyzed in detail with Q-, G-, R-, and C-banding techniques, and the rearrangement was identical to the one previously reported (SEUÁNEZ et al., 1976). This is a complex rearrangement involving three breaks and an *inverted insertion* (PARIS

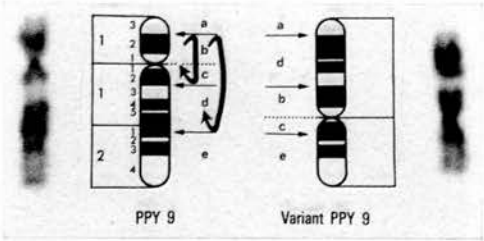


*Fig. 2.* Photograph and diagram of the G-banding patterns of the normal (left) and variant chromosome (right). Straight arrows represent the break points. Curved arrows represent the two pericentric inversions, one inside the other.

CONFERENCE, 1971). Break points were identified by both G- and R-banding techniques, and the rearrangement can be viewed as two pericentric inversions, one inside the other, resulting in the rearranged chromosome of the form *inv ins*(9)(p13q13q21); the variant chromosome 9 could then be described in detail as pter→p13::q21→q13::p13→q13::q21→qter (see fig. 2).

Q- and C-banding performed on the same cells showed that both the normal and the variant chromosomes 9 are the only chromosomes of the complement not having a positive centromeric C-band (see fig. 3). The significance of this finding is unclear.

## *Discussion*

Previous reports on the chromosomes of *Pongo pygmaeus* have identified one chromosome as identical to chromosome 12 in man in general morphology and with regard to G- (or R-) and Q-banding patterns (TURLEAU et al., 1972; DE GROUCHY et al., 1973; DUTRILLAUX et al., 1975; TURLEAU et al., 1975). This chromosome has been designated as chromosome 9 in the karyotype of *Pongo pygmaeus* (PARIS CONFERENCE, 1971, SUPPLEMENT, 1975). The first evidence of a variant chromosome 9 appeared in a report by LUCAS et al. (1973), where the chromosome was classified as a member of pair 7 and showed a similar G-banding pattern to the variant chromosome 9 described here. DUTRILLAUX et al. (1975) have also reported a variant chromosome 9 (No. 12 in their classification) in an orangutan with a male phenotype and a 48,XX chromosome constitution; the rearrangement was considered as an insertion

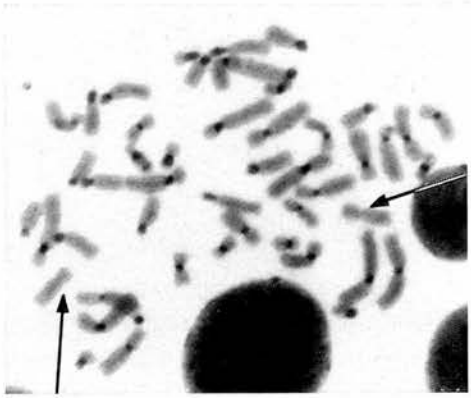

*Fig. 3.* C-banded metaphase in an heterozygous carrier showing chromosome pair 9. Note the absence of a positive C-banded region at the centromere. The same chromosomes were previously identified with Q-banding.

of the segment q11q22 in p22. TURLEAU et al. (1975) have reported a variant chromosome 9 (No. 12 in their classification) in a family of Bornean orangutans, and the rearrangement was interpreted as an insertion of the centromere, *ins cen*(12)(q213). Finally, a variant chromosome 9 was also reported by us (SEUÁNEZ et al., 1976) in a male orangutan of Sumatran origin. In our own study the rearrangement was interpreted as a pericentric inversion and a reciprocal, direct transposition of the terminal segments of both arms; such a rearrangement may also be considered as being derived from two pericentric inversions, one inside the other, as mentioned above.

The significance of these variant chromosomes was not entirely understood, since it was not clear whether they were due to complex rearrangements occurring sporadically in isolated specimens or in close family groups. This report clearly shows that a variant chromosome 9 was found in 12 orangutans, 4 of Bornean and 8 of Sumatran origin. Within each population group, the animals of the adult generation are not apparently closely related and were caught wild in the same islands at different periods. Most of these adult animals, whether male or female, are fertile, and their fertility does not appear to be impaired by the fact that they may carry one or two of the variant chromosomes 9. Moreover, a detailed comparison of all these reported variants (LUCAS et al., 1973; DUTRILLAUX et al., 1975; TURLEAU et al., 1975) indicates that all describe an identical rearrangement involving chromosome 9, so that this must be

a common type of variant chromosome with a high incidence in populations of *Pongo pygmaeus*.

A genetic trait which occurs in a breeding population with a higher incidence than would be maintained by recurrent mutation may be defined as a polymorphism (FORD, 1940). The complex rearrangement originating this new chromosome type must have occurred in the ancestral stock, before the original population of *Pongo* spread into both Sumatra and Borneo. These two islands are separated by the South China Sea, and it is believed (CROSBY, personal communication) that their separation occurred around 8000 years ago, so that both populations of *Pongo* must have been effectively isolated for a period encompassing roughly 500 generations. The fact that the rearrangement is both ancient and widespread suggests that it has been maintained in the population as a balanced type of polymorphism. For this to happen, we should expect that there is no selective disadvantage to heterozygous carriers, as indicated by our own results and by previous reports (TURLEAU et al., 1975). We should note, however, that the rearrangement would, in the heterozygous state, result in the formation of two inversion loops at the first meiotic division, as illustrated in fig. 4. Single, and most double, cross-overs in such a bivalent would result in the formation of unbalanced products of the duplication-deficiency or dicentric type. However, if crossing-over was confined to the terminal segments of the chromosomes (segments *a* and *e* in fig. 4), this would not affect the production of balanced gametes, and the situation would be compatible with normal fertility. We know that in the equivalent chromosome in man (No. 12) most chiasmata are indeed found in the terminal segments of the chromosome arms (CHANDLEY, unpublished observation). If this were the case in *Pongo,* and if chiasma position is an accurate reflection of cross-over site, then this preferential location of chiasmata might well contribute to the maintained fertility of the heterozygous carriers. In this context it is pertinent to note that sperm samples were obtained from one heterozygous carrier (S.I.16) who has not yet a proven fertility, and these contained a very high percentage of regular-sized and shaped spermatozoa in similar proportions as in sperm samples from S.I.17, a homozygous carrier of the variant chromosome, who had a proven fertility.

In mammals, in general, Robertsonian translocations are frequently found as chromosome polymorphisms (WHITE, 1973), but other types of polymorphic chromosome rearrangements are less frequent. There is evidence, however, that pericentric inversions may be polymorphic in


*Fig. 4.* Theoretical configuration of bivalent 9 in a heterozygous carrier. Regions *a* to *e* are the regions illustrated in fig. 2. Cross-overs at regions *a* and *e* will result in balanced products. A single cross-over at region *b* or *c* would result in unbalanced products of the duplication-deficiency type. A single cross-over at region *d* would result in unbalanced products, carrying dicentric chromosomes and acentric fragments.

populations of the deer mouse (OHNO et al., 1966; ARAKAKI et al., 1970), plains wood rat (BAKER et al., 1970), South American populations of *Rattus rattus* (BIANCHI and PAULETTE-VANREL, 1969), mountain voles (GILEVA and POKROVSKI, 1970), African pygmy mice (MATTHEY and JOTTERAND, 1970; JOTTERAND, 1972), and the European shrew (FORD and HAMERTON, 1970).

In the Hominoidea, it has been suggested (DE GROUCHY et al., 1973) that pericentric inversions were the major kinds of rearrangements involved in chromosome evolution within the group, and clear examples of homologies between chromosomes differing by an inverted pericentric region are evident (PARIS CONFERENCE, 1971, SUPPLEMENT, 1975). The presence of a major chromosome rearrangement as a polymorphic feature in a hominoid population is thus a striking and unusual finding of particular interest. It is relevant, therefore, to note here not only that chromosomes 9 and 12 of the orangutan and man show a similar over-all morphology and banding pattern but that these differ from the homologous chromosome pairs in the gorilla and chimpanzee (PTR10 and GGO10) by a single pericentric inversion. The new inversions described in the present work have, in fact, resulted in a chromosome that has a similar arm ratio to chromosome 10 of the gorilla and chimpanzee, but the double nature of the inversion gives rise to a somewhat different banding profile.

In man, obvious pericentric inversions involving whole, or parts of, C-band regions occur with relatively high frequencies in at least two chromosomes in the complement (BUCKTON et al., 1976). These human inversions, however, are small, appear to be confined to regions occupied by constitutive heterochromatin—regions considered to have a low genetic activity—and are not, therefore, comparable to the situation described here in *Pongo pygmaeus*. On the other hand, there are reports of larger pericentric inversions in man which involve euchromatic chromosome regions, but these are often associated with pathological conditions in carriers. In some less common cases, these larger pericentric inversions have been found to be transmitted by phenotypically normal heterozygotes who showed normal fertility (JACOBS et al., 1967; WEITKAMP et al., 1969; CRANDAL and SPARKES, 1970; BETZ et al., 1974; DE LA CHAPELLE et al., 1974; JACOBS et al., 1974), but the incidence of chromosomes with such inversions in human populations is extremely low. Moreover, in all these cases ascertainment was through some kind of clinically abnormal condition, and in only one (BETZ et al., 1974) was the inversion present in the homozygous state. Thus in man, the most well studied of the hominoids, none of the pericentric inversions that involve significant amounts of euchromatic material can be considered to be fixed balanced polymorphisms in the population. Whether polymorphic rearrangements of the type uncovered in our study of the orangutan are to be found in other hominoid species, however, is an open question.

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## *Appendix*

### *Registry of the animals*

*Bornean*

| | | | | | |
|---|---|---|---|---|---|
| B.I.1 | Jack | (Bristol) | B.II.1 | James | (Bristol) |
| B.I.2 | Jill | (Bristol) | B.II.2 | Popi | (Yerkes) |

| | | | | | |
|---|---|---|---|---|---|
| B.I.3 | Abang | (Bristol) | B.II.3 | Teriang | (Yerkes) |
| B.I.4 | Dyang | (Bristol) | | | |
| B.I.5 | Padang | (Yerkes) | | | |
| B.I.6 | Paddi | (Yerkes) | | | |
| *Sumatran* | | | | | |
| S.I.1 | Sampit | (Yerkes) | S.II.1 | Kanting | (Yerkes) |
| S.I.2 | Jowata | (Yerkes) | S.II.2 | Santing | (Yerkes) |
| S.I.3 | Bagan | (Yerkes) | S.II.3 | Jinjing | (Yerkes) |
| S.I.4 | Lada | (Yerkes) | S.II.4 | Tukan | (Yerkes) |
| S.I.5 | Dyak | (Yerkes) | S.II.5 | Sah | (Yerkes) |
| S.I.6 | Tupa | (Yerkes) | S.II.6 | Anak | (Yerkes) |
| S.I.7 | Bali | (Yerkes) | S.II.7 | Chebek | (Yerkes) |
| S.I.8 | Tuan | (Yerkes) | S.II.8 | Ayer | (Yerkes) |
| S.I.9 | Ini | (Yerkes) | S.II.9 | Jiran | (Yerkes) |
| S.I.10 | Datu | (Yerkes) | S.II.10 | Loklok | (Yerkes) |
| S.I.11 | Sungei | (Yerkes) | S.II.11 | Patpat | (Yerkes) |
| S.I.12 | Lipis | (Yerkes) | S.II.12 | Petala | (Yerkes) |
| S.I.13 | Sibu | (Yerkes) | S.II.13 | Kesa | (Yerkes) |
| S.I.14 | Henry | (Bristol) | S.II.14 | Lunak | (Yerkes) |
| S.I.15 | Ann | (Bristol) | S.II.15 | Oscar | (Bristol) |
| S.I.16 | Dinding | (Yerkes) | S.II.16 | Henrietta | (Bristol) |
| | | | S.II.17 | Julitta | (Bristol) |
| *Hybrid matings* | | | | | |
| S.I.17 | Kampong | (Yerkes) | H.I.1 | Kanak | (Yerkes) |
| B.I.6 | Paddi | (Yerkes) | H.I.2 | Bebas | (Yerkes) |
| B.I.7 | Kyan | (F. Walton Zoo) | | | |
| S.I.18 | Potts | (F. Walton Zoo) | | | |

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# Fluorescent (F) bodies in the spermatozoa of man and the great apes

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*Abstract*

Mature spermatozoa of the chimpanzee *(Pan troglodytes),* the gorilla *(Gorilla gorilla),* and the orangutan *(Pongo pygmaeus)* were stained with quinacrine dihydrochloride. Fluorescent (F) bodies were visualized in the spermatozoa of the chimpanzee and gorilla but were absent in the orangutan, in which there is no brilliant fluorescence in any chromosome. The F bodies appeared to be randomly located in the sperm heads of these two species, as they usually are in human spermatozoa. However, the proportion of sperm showing one or more F bodies in the chimpanzee and gorilla was not comparable to what is usually found in man. The F bodies in the chimpanzee presumably represent brilliant regions in the autosomes, since the Y chromosome has no brilliant fluorescence in this species. This is contrary to man, in which the F body is an useful indicator of the Y chromosome. In the gorilla, the F bodies probably correspond to both the Y chromosome and to some brilliant regions in the autosomes.

The introduction of fluorescent dyes to stain human chromosomes (CASPERSSON et al., 1970, 1971) has allowed identification of homologous chromosomes by their banding patterns, revealed by differential staining along the chromatids. It has also shown that some chromosome regions may fluoresce very intensely, standing out brilliantly in relation to the rest of the human complement. These regions comprise the distal end of the Y chromosome, the short arm-satellited areas of the acrocentric chromosomes, and the centromeric areas of chromosomes 3 and 4 (PARIS CONFERENCE, 1971, and fig. 1). All these regions are polymorphic (see BUCKTON et al., 1976); those at the autosomes may vary in their size

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and brightness, whereas that of the Y chromosome seems to vary only in size (BOBROW et al., 1971). Except for rare instances when the Y chromosome is very short and its brilliant fluorescent region absent, this chromosome can be visualized in interphase cells (PEARSON et al., 1970), in meiotic series (PEARSON and BOBROW, 1970), and in spermatozoa (BARLOW and VOSA, 1970) as a brilliant fluorescent spot, the F body. This has permitted the recognition of X- and Y-bearing spermatozoa (SUMNER et al., 1971); since a small proportion of spermatozoa show two F bodies, it is possible that they may contain two Y chromosomes (see SUMNER and ROBINSON, 1976).

The chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*) also show brilliant fluorescent regions in some of their chromosomes (PEARSON et al., 1971; DUTRILLAUX, 1973; EGOZCUE et al., 1973; LEJEUNE et al., 1973; LIN et al., 1973; PEARSON, 1973; MILLER et al., 1974; PARIS CONFERENCE, 1971, SUPPLEMENT, 1975). In the chimpanzee, brilliant

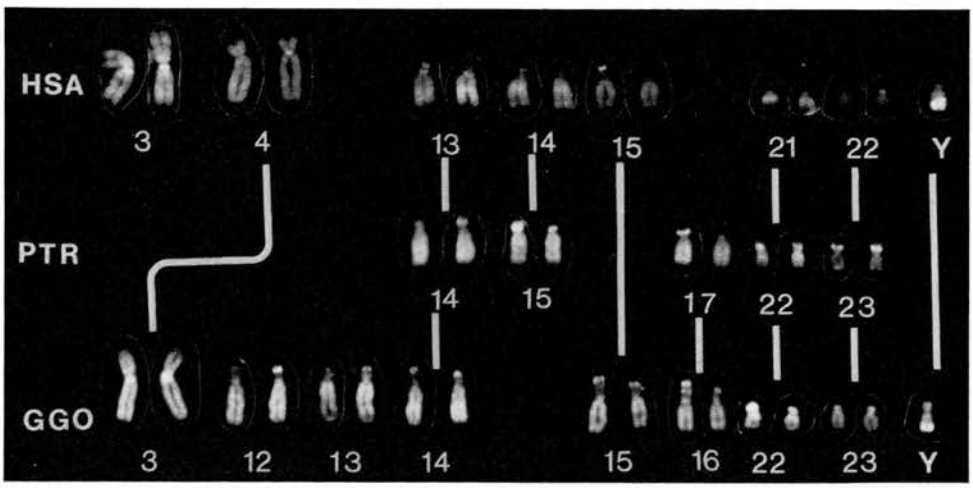


*Fig. 1.* Chromosomes of man (HSA), the chimpanzee (PTR), and the gorilla (GGO) showing brilliant fluorescent regions (PARIS CONFERENCE, 1971, SUPPLEMENT, 1975). Presumptive homologies are indicated by vertical lines. Notice that in man the average-size brilliant region of the Y chromosome is larger than those of the autosomes. In the chimpanzee, the autosomal brilliant regions are usually greater than those of the human autosomes. In the gorilla, the brilliant region of the Y is usually smaller than the average-sized region of the human Y chromosome. Notice that some regions, such as the autosomes in the gorilla, may be as large as that of the Y chromosome (compare GGO3, GGO22, and GGOY).

fluorescence is restricted to the autosomes (pairs 14, 15, 17, 22, and 23). The Y chromosome, a small submetacentric, stains very palely (fig. 1). But the gorilla shows brilliant fluorescence not only in some autosomes (pairs 2, 12, 13, 14, 15, 16, 22, and 23 [PARIS CONFERENCE, 1971, SUPPLEMENT, 1975]) but also at the terminal segment of the Y chromosome (fig. 1). In the orangutan, none of the chromosomes fluoresce brilliantly.

In this paper we describe observations of the spermatozoa of *Pan, Gorilla,* and *Pongo* after staining with quinacrine dihydrochloride. The results are compared with those obtained with human spermatozoa.

## *Materials and methods*

Semen was obtained from three adult male chimpanzees (two of which were presumably fertile) in Edinburgh Zoo following spontaneous masturbation and from one fertile adult male gorilla and two fertile orangutans in the Yerkes Primate Center, Atlanta, Georgia, by electroejaculation, using the technique of WARNER et al. (1974).

In all cases, 1 ml of the fresh semen was resuspended in 6 ml of 0.9 % NaCl and centrifuged at 2000 rpm for 5 min. This procedure was repeated twice, and after the third centrifugation the remaining pellet was resuspended gently in 6 ml of fixative (methanol:acetic acid 3:1) and allowed to fix at room temperature for 10 min. It was then centrifuged at 2000 rpm for 5 min and the supernatant discarded. This procedure was repeated twice, and after the third centrifugation the final pellet was resuspended in 1 ml of the remaining fixative; air-dried preparations were then made on precleaned slides. Spermatozoa were stained with quinacrine dihydrochloride, following the technique described by ROBINSON and BUCKTON (1972), and later observed under blue light. A fluorescent spot was considered to be an F body if its size and brightness was comparable to or greater than that of the F body in human spermatozoa.

Chromosome nomenclature and the presumptive homologies between the chromosomes of man and the Hominoidea are those approved by the PARIS CONFERENCE (1971), SUPPLEMENT (1975).

## *Results*

A detailed study on the morphology, head size, and shape of the spermatozoa of the great apes has appeared (see MARTIN et al., 1975), and our general observations on sperm morphology, with different techniques, confirm these findings.

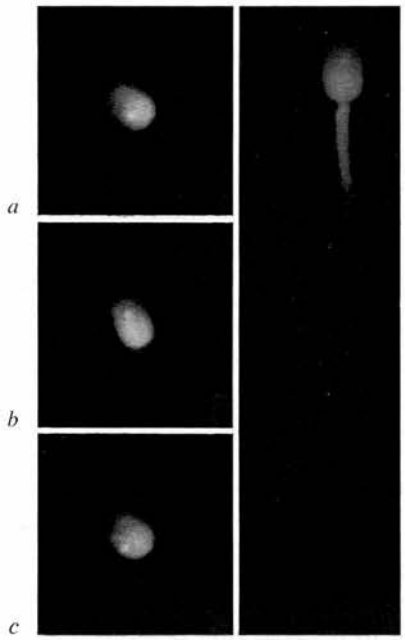


*Fig. 2.* Spermatozoa of chimpanzee *(Pan troglodytes)* after quinacrine fluorescence. *a–c.* Sperm head with visible F body. *b.* Sperm head with visible F body in the boundary between the dense-staining area of the head and the pale area of the acrosomal cap. *d.* Entire spermatozoon showing the thick midpiece which stains as intensely as the head.

Chimpanzee spermatozoa were very homogeneous in morphology. The sperm head in the chimpanzee was elongated, with its longitudinal axis approximately twice as long as its transverse axis (fig. 2), and it could be easily distinguished from human spermatozoa. Chimpanzee sperm heads appeared more intensely stained than the average staining intensity of the human sperm head (fig. 2*b*), and fluorescence was brightest at the central region above the attachment of the tail. The midpiece in the chimpanzee was also longer and wider than in man, and it fluoresced as intensely as the sperm head (fig. 2*d*), whereas in man the midpiece is usually negatively stained under similar conditions. Distinct F bodies were observed, and their position did not show any preferential location in the sperm head, although those located in the boundary between the dense area of the head and the more distal area below the acrosomic cap were more contrasted and easier to photograph (fig. 2*b*). The number of cells with F bodies and their number per cell are shown in table I.

*Table I.* Distribution of F bodies in the spermatozoa of the great apes.

| Number of F bodies | Chimpanzee 1 | Chimpanzee 2 | Chimpanzee 3 | Gorilla | Orangutan |
|---|---|---|---|---|---|
| 0 | 419 (83.80 %) | 68 (34.0 %) | 156 (78.0 %) | 203 (40.60 %) | 200 (100 %) |
| 1 | 73 (14.60 %) | 74 (37.0 %) | 31 (15.5 %) | 181 (36.20 %) | – |
| 2 | 8 (1.60 %) | 52 (26.0 %) | 13 (6.5 %) | 93 (18.6 %) | – |
| 3 | – | 5 (2.5 %) | – | 22 (4.40 %) | – |
| 4 | – | 1 (0.5 %) | – | 1 (0.2 %) | – |
| Total | 500 (100 %) | 200 (100 %) | 200 (100 %) | 500 (100 %) | 200 (100 %) |
| Cells with even numbers of F bodies (0, 2, 4) | 427 (85.40 %) | 121 (60.50 %) | 169 (84.50 %) | 297 (59.40 %) | |
| Cells with odd numbers of F bodies (1 and 3) | 73 (14.60 %) | 79 (39.50 %) | 31 (15.50 %) | 203 (40.60 %) | |
| Chi-square (df = 1) | 250.63 | 8.82 | 95.22 | 17.672 | |
| Probability | $<0.0001$ | $<0.01$ | $<0.0001$ | $<0.0001$ | |

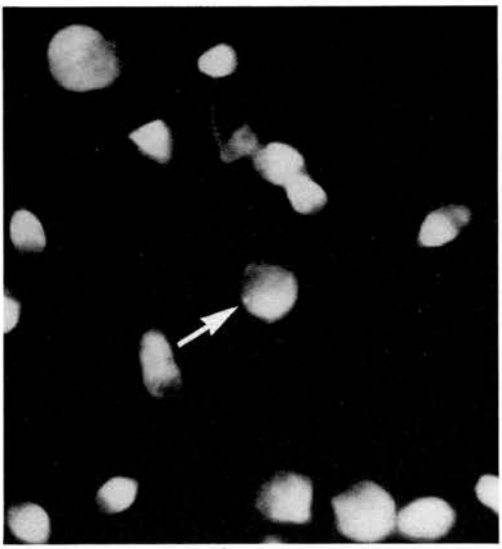

*Fig. 3.* Spermatozoa of the gorilla *(Gorilla gorilla)* after quinacrine fluorescence. Notice the obvious variation in morphology of the heads. The arrow points to a round-headed spermatozoon with a visible F body.

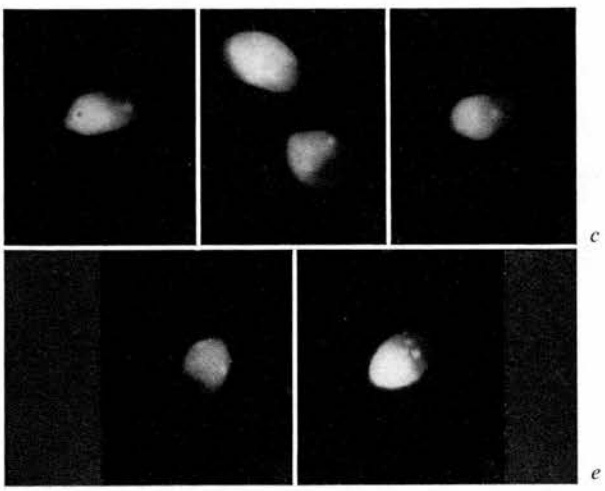


*Fig. 4.* Spermatozoa of the gorilla after quinacrine fluorescence. Notice the variable location of the F bodies in the sperm head. Spermatozoa in *a, c, d,* and *e* show an oval-shaped head of uniform size which corresponds to the modal cell type in the gorilla; they are identical in morphology to the modal type in man.

In the gorilla, we found a wide range of variation in sperm morphology, both in size and shape, which resembled the usual pleomorphism found in human spermatozoa. The most common abnormality was that affecting the head shape (fig. 3), and the modal type in the gorilla had a rather oval-shaped head which closely resembled the modal cell type in human spermatozoa. The midpiece was negatively stained. Distinct F bodies were identified in the sperm heads of the gorilla (fig. 4*a–e*). The location of F bodies varied considerably in the sperm head and appeared to be random. F bodies could clearly be observed against the pale background below the acrocentric cap (fig. 4*a*), in the boundary between the dense staining area of the head and pale distal end (fig. 4*c*), or even below the boundary between these two regions (fig. 4*b*). Sperm heads with two distinct F bodies were common (see table I), and here again they seemed to be randomly located in any region of the sperm head (compare fig. 4*d* and *e*). Spermatozoa with more than two F bodies were also identified, although they were usually in different planes of the sperm head, which made them impossible to photograph. The number of cells with F bodies and their number per cell are shown in table I.

In the orangutan, the sperm heads were regular in size and shape. They were clearly larger than in man, chimpanzee, and gorilla, and they were rounder and broader. The intensity of fluorescence was pale (PARIS CONFERENCE, 1971), and it decreased gradually from the center of the sperm head to the periphery. The midpiece was negatively stained. No visible F bodies were detected.

## *Discussion*

The detection of F bodies in the spermatozoa of the chimpanzee and the gorilla and their absence in the orangutan was not unexpected in view of the quinacrine banding patterns of these species (fig. 1). In man, the brilliant fluorescent regions which may be present in some autosomes are very rarely as large as the brilliant region in the Y chromosome and are unlikely to show as a distinct F body. The brilliant region in the human Y chromosome is usually visible, although in somewhat less than 50 % of spermatozoa (PEARSON and BOBROW, 1970; SUMNER et al., 1971; PAWLOWITSKI and PEARSON, 1972; GERAEDTS and PEARSON, 1976). In the chimpanzee, the Y chromosome has no brilliant fluorescence, so the F bodies are presumably due to autosomal material (fig. 1).

If there were any single heterozygous brilliant region in any animal capable of showing constantly as an F body (call it $F_1$, say) we would expect that 50 % of spermatozoa would show it. Assuming that all other F bodies showed independently of $F_1$, then the number of spermatozoa containing $F_1$ and *n* other F bodies should be the same as the number *not* containing $F_1$ but containing *n* other F bodies. Since this should hold for all values of *n,* the number of sperm containing 0, 2, 4, ..., F bodies (even class) should equal the number containing 1, 3, 5, ..., F bodies (odd class). Since the even class is significantly ($P<0.01$) greater ($\chi^2$ test) than the odd class, the original hypothesis can therefore be ruled out.

Less than 25 % of spermatozoa showed F bodies in chimpanzees 1 and 3 (see table I), whereas 76 % of spermatozoa showed F bodies in chimpanzee 2. In chimpanzees 1 and 3 we can rule out the hypothesis that any single brilliant region is capable of showing constantly in spermatozoa, since in these two animals the proportion of cells with no F bodies (0 class) is significantly above 50 %. In chimpanzee 2, in which the 0 class is only 34 %, we can also rule out the hypothesis, by comparing the "even class" with the "odd class". Since the even class is significantly higher than the odd class ($P<0.01$), we may conclude that none of the brilliant autosomal regions in this animal which might be visible in spermatozoa are able to show constantly as a F body. The individual differences between animals might well reflect individual variations in the brilliant autosomal regions, which are known to be polymorphic in the chimpanzee (LIN et al., 1973).

The situation in the gorilla is complicated by the presence of fluorescent regions on both the Y chromosome and some autosomes. The incidence of spermatozoa with two F bodies was 18.6 %, compared with 1.25 % in man (SUMNER et al., 1971). Nondisjunction of the Y chromosome is unlikely to account for this high incidence, nor could it explain the spermatozoa with three and four F bodies. Using the same type of frequency distribution analysis as applied to the chimpanzee, the fact that 40 % of spermatozoa show no visible F body rules out the possibility of a single homozygous fluorescent region. Since there was a significantly higher preponderance of cells with an even number of F bodies, this once again suggests that no single brilliant region, whether on the Y or on an autosome, was always visible. Since the highest number of F bodies detected was four, at least three autosomes in addition to the Y chromosome can apparently be visualized in some spermatozoa.

The location of F bodies in the sperm heads of the chimpanzee and

gorilla appeared to be randomly distributed. This observation in these two species resembles the findings of GERAEDTS and PEARSON (1976) after staining human spermatozoa with both G-11 and atebrine. This combined procedure allowed identification of two chromosomes (the 9 and the Y), and their location in the sperm head appeared to be randomly located. A similar conclusion was reached by us (SEUÁNEZ et al., 1976) using hybridization in situ to cRNA III in human meiotic preparations coupled with quinacrine fluorescence, giving no indication that chromosomes in human spermatozoa might have a constant orientation.

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# Morphological abnormalities in spermatozoa of man and great apes

HUMAN semen has long been known to differ from that of any other mammal in the high proportion of abnormal spermatozoa that it contains[1]. But there has been almost no opportunity to compare human spermatozoa with those of man's closest living relatives, the chimpanzee (*Pan troglodytes*), the pygmy chimpanzee (*Pan paniscus*), the gorilla (*Gorilla gorilla*) and the orangutan (*Pongo pygmaeus*); these are all now endangered species, and the few animals in zoos are not available for experimentation. Recently, workers at the Yerkes Regional Primate Research Centre, where all four species are maintained, have perfected a technique for obtaining semen from these rare animals by electroejaculation[2]. Preliminary studies have suggested that marked morphological similarities exist only between the spermatozoa of the gorilla and man[3]. We examined these similarities and differences in more detail, in the hope that spermatozoal morphology might provide some taxonomic clues about our affinities to the great apes.

Table 1 shows that there is a similar proportion of 'normal' (modal) and abnormal forms in the ejaculate of men and gorillas; morphologically, the 'normal' forms of the two species are indistinguishable (Fig. 1). Chimpanzee spermatozoa are very different in appearance to human or gorilla spermatozoa, although very uniform in shape; they seem to be identical in all aspects to the spermatozoa of the pygmy chimpanzee. The spermatozoa of the orangutan are morphologically distinct from all the other species, although once again extremely uniform in shape.

Measurements of total dry mass were made with an integrated interferometer (Vickers M-86) for a random selection of 50–100 spermatozoa from each individual in each of the five species. In man and gorilla the dry mass distribution was bimodal with two distinct non overlapping peaks, whereas in the chimpanzees and the orangutan it was unimodal (Fig. 2). When arbitrary units of dry mass were used, the mean value of the major peak in man was 730 compared with 960 in the gorilla. In both species the minor peak represented a dry mass approximately twice that of the major peak. These results are similar to those reported for Feulgen-stained human spermatozoa[4], and suggest that the two groups represent haploid and diploid spermatozoa. The percentage of diploid spermatozoa in man and gorilla was estimated using a larger sample; 21 diploids were found in 2,000 human spermatozoa (1.05%) and 37 in 2,408 gorilla spermatozoa (1.53%). This difference was not statistically significant ($P > 0.10$). Human spermatozoa had the lowest mean haploid dry mass, and gorilla spermatozoa the highest (Table 2). By extracting DNA with trichloroacetic acid and remeasuring dry mass it could be shown that total dry mass was proportional to DNA content in *Homo sapiens*, *Pan troglodytes*, *Gorilla gorilla* and *Pongo pygmaeus* (Fig. 3). Statistical analysis (Table 2) revealed no significant differences in dry mass between individuals within a species, but marked differences between species. Although human spermatozoa are morphologically most similar to those of the gorilla, in terms of DNA content they resemble those of the chimpanzees. Gorilla spermatozoa showed the greatest intra-individual variance in haploid DNA content.

Diploid DNA estimates in the somatic cells of the great apes and man have revealed *Homo sapiens*, < *Gorilla gorilla*, < *Pan troglodytes*, < *Pongo pygmaeus*[5] which does not correspond with our haploid estimates of *Homo sapiens* < *Pan troglodytes*, < *Pongo pygmaeus*, < *Gorilla gorilla*. Man has a diploid chromosome number of 46 compared with 48 in all great apes, and less constitutive heterochromatin and brilliant fluorescent chromatin than the chimpanzee and the gorilla[6]. The overall amount of satellite DNA sequences in man seems to be lower than in the great apes[7], and presumably all these factors contribute to the low DNA content of human cells.

It has been suggested[8] that the pleiomorphism of human spermatozoa may be a consequence of testicular damage caused by clothing-induced hyperthermia. The fact that the spermatozoa of gorillas living in outdoor cages are just as pleomorphic, and show an even greater variability in haploid DNA content, tends to refute this argument. Furthermore, the great variability in morphology of human spermatozoa, is contrast to the lack of variability in haploid DNA content, means that morphologically abnormal human spermatozoa are not necessarily genetically defective. The percentage of morphologically abnormal forms is increased in infertile men, however, sometimes reaching very high proportions in cases of abnormal chromosome constitution[9]. It has even been suggested that fertilisation of a defective egg by diploid spermatozoa will give rise to a hydatidiform mole[10], and that 40% of triploid embryos are a result of fertilisation of a normal egg by a diploid spermatozoon[11].

Spermatozoal morphology has been shown to be a useful taxonomic guide in assessing relationships between other species[12]. There is already evidence to suggest that man and gorilla are more closely related than any of the other great apes, as judged by their chromosomal karyotypes[13]. Our data on the morphology of their spermatozoa lends further support to this conclusion.

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**Table 1** Morphology of spermatozoa of man and the great apes

| | | *Homo sapiens* (4 individuals) | *Pan troglodytes* (3 individuals) | *Pan paniscus* (1 individual) | *Gorilla gorilla* (2 individual) | *Pongo pygmaeus* (2 individuals) |
|---|---|---|---|---|---|---|
| Normal | Modal | 73.0 | 95.5 | 98.0 | 71.0 | 98.5 |
| Abnormal | Large head | 2.0 | 0.3 | – | 2.3 | – |
| | Small head | 0.5 | – | 0.5 | 0.8 | – |
| | Tapered head | 0.3 | 0.2 | – | 1.0 | – |
| | Dense-staining | 0.6 | 0.2 | 0.5 | 4.5 | – |
| | Vacuolated head | 2.4 | 3.0 | 1.0 | 1.3 | 0.3 |
| | Irregularly shaped | 18.4 | 0.2 | – | 16.0 | 0.8 |
| | Multiple heads | 0.5 | – | – | 0.5 | – |
| | Abnormal midpiece | – | 0.3 | – | 0.5 | – |
| | Cytoplasmic droplets | – | – | – | 0.3 | – |
| | Immature cells | 2.3 | 0.3 | – | 2.0 | 0.5 |
| | Total | 100.0 | 100.0 | 100.0 | 100.2 | 100.1 |

except for one ~~male~~ ~~orang-utan~~ & Pongo pygmaeus

Semen ~~samples~~ were obtained from normal healthy individuals of proven fertility. In man ~~(four individuals)~~ and in *Pan troglodytes* ~~(three individuals)~~ samples were obtained by masturbation. In *Pan paniscus* ~~(one individual)~~, *Pongo pygmaeus* ~~(two individuals)~~ and *Gorilla gorilla* ~~(two individuals)~~ samples were obtained by electroejaculation as described by Warner *et al.*[2]. Semen samples were washed in 0.9% saline and fixed in methanol:acetic acid, 3:1 as described by Sumner[4]. Some of these slides were stained with Papanicolaou's stain and observed under the light microscope: 200 cells were scored per individual and all results of Table 1 are expressed as percentages.

Papanicolaou's



**Table 2** Comparisons of total dry mass of spermatozoa of the great apes and man

| Species | Means | Difference | Approximate 95% confidence limits Assumption A Lower | Assumption A Upper | Assumption B Lower | Assumption B Upper |
|---|---|---|---|---|---|---|
| Chimpanzee–human | 810.0 – 742.3 | +67.7 | +21.8 | +113.6 | +41.8 | +93.6 |
| Orangutan–human | 871.6 – 742.3 | +129.3 | +73.0 | +185.6 | +97.6 | +161.0 |
| Gorilla–human | 965.2 – 742.3 | +222.9 | +166.4 | +279.5 | +190.7 | +255.2 |
| Orangutan–chimpanzee | 871.6 – 810.0 | +61.6 | +5.3 | +117.9 | +29.9 | +93.3 |
| Gorilla–chimpanzee | 965.2 – 810.0 | +155.2 | +98.7 | +211.8 | +123.0 | +187.5 |
| Gorilla–orangutan | 965.2 – 871.6 | +93.6 | +28.5 | +158.9 | +56.6 | +130.7 |

The component of variance between individuals within a species is taken to be either 2,000 a.u.$^2$ (assumption A) or 1,000 a.u.$^2$ (assumption B).

Anovar of haploid dry-mass revealed (1) no significant differences between preparations from the same individual, (2) that the intra-individual variance components for man, chimpanzee and orangutan did not differ significantly (pooled estimate = 4,949.3 with 485 d.f.), but were significantly less than for gorilla (11,606.7 with 197 d.f.). Confidence intervals were constructed using these estimates and taking values of the inter-individual intra-species component to be either 2,000 a.u.$^2$ (assumption A) or 1,000 a.u.$^2$ (assumption B). The assumptions correspond to situation in which one individual in 100 can be expected to differ from its species mean by the equivalent of approximately two (A) or one (B) medium-sized chromosomes respectively. The implied degree of aneuploidy suggests that these are overestimates, as are the widths of the corresponding confidence intervals.

**Fig. 1** The spermatozoa of *Pan paniscus* (*a*); *Pan troglodytes* (*b*); *Pongo pygmaeus* (*c*); *Homo sapiens* (*d*); and *Gorilla gorilla* (*e*) photographed under Nomarski interference. Scale bar, 10 ßm.

**Fig. 2** The estimations of total dry mass (TDM) were obtained with a Vickers M-86 integrated microinterferometer. This instrument measures the optical path difference (o.p.d. = refractive index × thickness) in arb. units. The area of the object (the sperm head only) was selected using an electronic masking system. Measurements were obtained with a X 75 n.a. 1.1 water immersion objective and preparations were measured while immersed in distilled water. All semen samples were treated as specified in the legend of Table 1, except that measurements were done on unstained preparations. 50 cells were measured per individual using two slides (25 measurements in each), except in the gorillas (100 cells; 50 measurements per slide). *a*, *Homo sapiens*; *b*, *Pan troglodytes*; *c*, *Pan paniscus*; *d*, *Pongo pygmaeus*; *e*, *Gorilla gorilla*.

(a.u.)

arbitrary units

delete

delete

**Fig. 3** To test whether TDM is proportional to DNA content, 10 spermatozoa from each of two individuals from each species (except *Pan paniscus*) were measured for TDM. After treatment with 5% trichloracetic acid at 90 °C to remove DNA, the same spermatozoa were relocated and measured for dry mass after extraction (DMAE). Slides were then stained with Feulgen and the same spermatozoa measured for residual DNA with the M-86 instrument in the microdensitometric mode. Only if no DNA was detected were the DMAE values considered valid. The minimum extraction time needed to remove all DNA was 15 min. in man and 30 min. in the great apes. The difference between TDM and DMAE represents the dry mass of extracted DNA (DNA-DM). The figure shows mean values of DNA-DM and TDM for each individual, and the estimated least-squares regression line. The latter passes close to the origin, suggesting that proportional changes in TDM are identical to those for DNA-DM. (The line should not of course be interpreted as a valid extrapolation beyond the range of the plotted points.) ○, *Homo sapiens*; □, *Pan troglodytes*; ▽, *Pongo pygmaeus*; , *Gorilla gorilla*.

◇ – (Diamond)



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