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A Note on the Karyotypes and Chromosome Associations of the Hylobatidae, with Special Reference to *Hylobates* (*Nomascus*) *concolor*

The somatic chromosomes, obtained from short term leukocyte cultures, were studied of four species of the Hylobatidae: *Hylobates lar, H. agilis, H.* (*Nomascus*) *concolor* and *Symphalangus syndactylus*. In accordance with earlier observations by others, the diploid chromosome numbers were found to be 44 in both *Hylobates lar* and *H. agilis*, 52 in *H. concolor* and 50 in *Symphalangus syndactylus*. The chromosome associations observed in metaphase spreads are clearly different in the three types of chromosome complements. In *Hylobates lar* and *H. agilis* associations are found between both members of the marked chromosome pair. In *Symphalangus syndactylus* the only two acrocentric elements of the karyotype, which are of medium size, associate frequently. In *H. concolor* finally, the members of three pairs of small acrocentrics are involved in chromosome associations. G-banding patterns (obtained by trypsin treatment) showed that in a male individual of this species also the small acrocentric Y chromosome sometimes participates in these associations. The evolutionary aspects of these observations are briefly discussed.

1. Introduction

Groves (1968) proposed a classification of the extant gibbons into a single genus, *Hylobates*, with three subgenera, *Hylobates*, *Nomascus* and *Symphalangus*. Fairly extensive chromosome studies in the Hylobatidae have shown, that this division in three groups is in accordance with the karyological characteristics. All hitherto studied forms of the subgenus *Hylobates* have nearly identical karyotypes with 44 chromosomes (Chu & Bender, 1961; Chiarelli, 1962, 1963, 1972; Bender & Chu, 1963; Hamerton *et al.*, 1963; Markvong, 1973). The single representative of the subgenus *Nomascus*, *Hylobates* (*Nomascus*) *concolor*, has a diploid chromosome number of 52 (Wurster & Benirschke, 1969; Markvong, 1973), while the siamang, *Hylobates* (*Symphalangus*) *syndactylus*, has 50 chromosomes (Klinger, 1963). The dwarf siamang, *Hylobates klossi*, originally arranged by Groves in the subgenus *Symphalangus*, appeared to possess a karyotype nearly identical to those of the forms of the subgenus *Hylobates* ($2n = 44$) (Hösli & Lang, 1970). At first sight, the chromosome complements of *Nomascus* and *Symphalangus* show more resemblance to each other than to the karyotype of *Hylobates*.

The karyotypes of the Hylobatidae are clearly distinct from those of the anthropoid apes, and especially the subgenus *Hylobates* karyologically resembles much more the Colobinae (Cercopithecidae), all representatives of which also have a diploid number of 44. This led Chiarelli (1968, 1973) to suggest a closer relationship between Hylobatidae and Cercopithecidae than between Hylobatidae and Pongidae.

One of the characteristics uniformly found in the karyotypes of the Cercopithecidae is the presence of a single pair of chromosomes with a broad secondary constriction, the so-called marked chromosome pair (Chiarelli, 1966; Egozcue & Vilarasau de Egozcue, 1967).

These elements are either submetacentric (*Macaca, Cercocebus, Papio*), metacentric (the Colobinae) or acrocentric (*Cercopithecus*). Independent from their structure, however, these elements are always found to be frequently involved in associations with each other in metaphase plates, as described by one of us (de Boer, 1972), a characteristic also noted by several other authors (Egozcue, 1968; Fernandez–Donoso *et al.*, 1970; Dzemilev, 1970; Ardito & Mortelmans, 1975).

In the Hylobatidae such marked chromosomes are only found in the representatives of the subgenus *Hylobates*, where they are metacentric as in the Colobinae. However, in *Symphalangus* nevertheless chromosome associations were found to occur between two satellited medium sized acrocentric elements (Klinger, 1963; de Boer, 1972). Since in *Nomascus* neither marked chromosomes, nor such medium sized satellited acrocentrics are found (Wurster & Benirschke, 1969; Markvong, 1973), it was considered to be of interest to restudy its karyotype, in order to detect possible associations of other chromosomes.

2. Material and Methods

Chromosome preparations were obtained from short term leukocyte cultures following usual techniques (de Boer, 1973*a*). Older preparations were already available of one female *Symphalangus syndactylus*, one male *Hylobates agilis* and one male and one female *H. lar*. New preparations were obtained of one male and two female specimens of *Hylobates* (*Nomascus*) *concolor*.

For chromosome identification by G-banding, part of these new preparations of *H. concolor* were treated with a 0·05% trypsin (Difco, 1:250) solution (pH 6·8) during 15–30 seconds, three times rinsed in distilled water, and stained in a 2% Giemsa solution in Sörenson's buffer (pH 6·8).

3. Results and Discussion

Generally our karyological results of the four species of Hylobatidae studied are in perfect agreement with the data already available from the literature (see Introduction), and a detailed description of the individual karyotypes therefore would be superfluous.

The chromosome complements of *Hylobates agilis* (Plate 1, a) and *H. lar* consist of 44 chromosomes and are nearly identical. All autosomes are biarmed, and most of them have low arm ratios. The X chromosome is a relatively large metacentric element, while the Y is of minute size and probably also biarmed. The marked chromosomes (pair 21) are nearly metacentric, with the constriction very close to the centromere in the short arm. Associations of these elements are frequently found. Both homologues are lying either side by side, with the constriction region close together (Plate 2, d & f), or crosswise with the constrictions in the center (Plate 2, e). These associations are similar to those found in all Cercopithecidae species with biarmed marked chromosomes (de Boer, 1972).

All except two of the 48 autosomes of *Symphalangus syndactylus* (Plate 1, b) are biarmed and nearly metacentric, with a gradual decrease in size between the longest and the shortest ones. Identification of individual pairs therefore is impossible, without application of banding techniques. According to Klinger (1963) the X chromosome is to be found among the longest metacentrics, while the Y is the smallest biarmed element in the complement (however, clearly larger than the Y of the subgenus *Hylobates*). The members

Plate 1. Representative karyograms of three species of Hylobatidae: (a) male *Hylobates agilis* ($2n = 44$); (b) female *Symphalangus syndactylus* ($2n = 50$, the X chromosomes have been tentatively chosen according to Klinger, 1963); (c) male *Hylobates* (*Nomascus*) *concolor* ($2n = 52$).

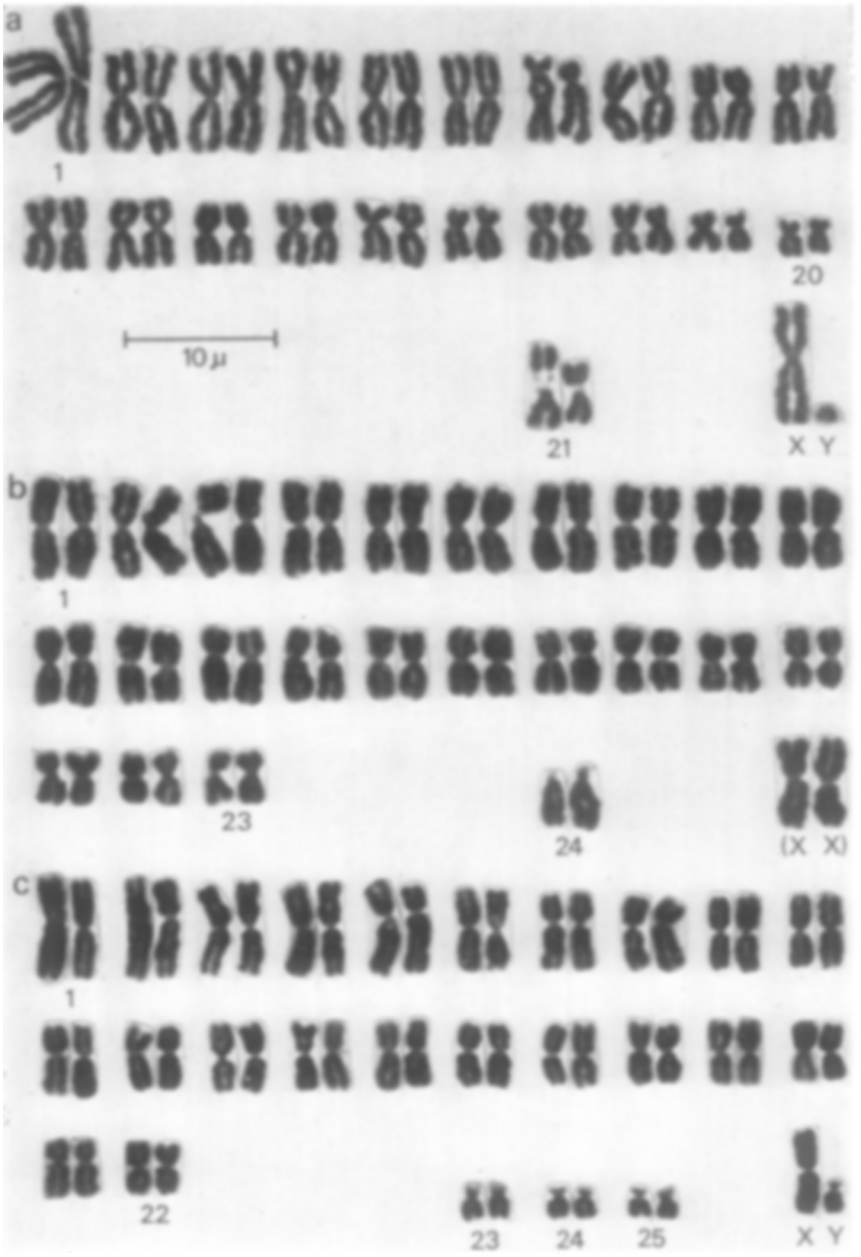


Plate 2. Details of metaphase spreads showing chromosome associations of three species of Hylobatidae: (a–c) *Symphalangus syndactylus;* (d–f) *Hylobates agilis;* (g–k) *Hylobates* (*Nomascus*) *concolor*. Magnification × 2000.

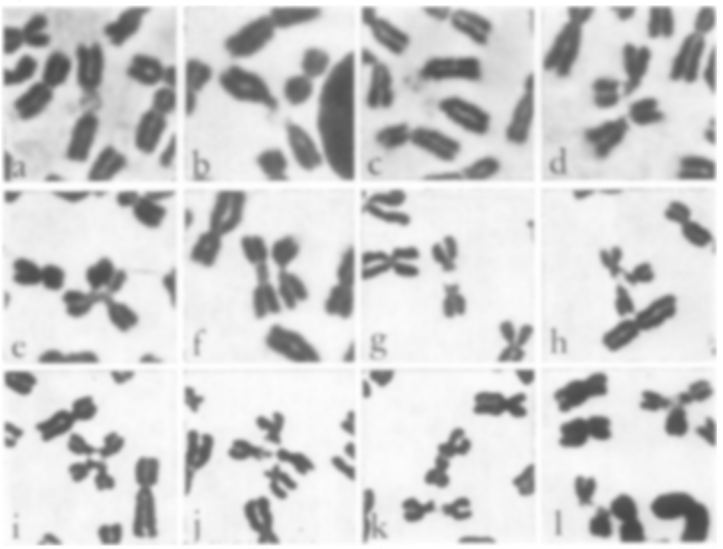
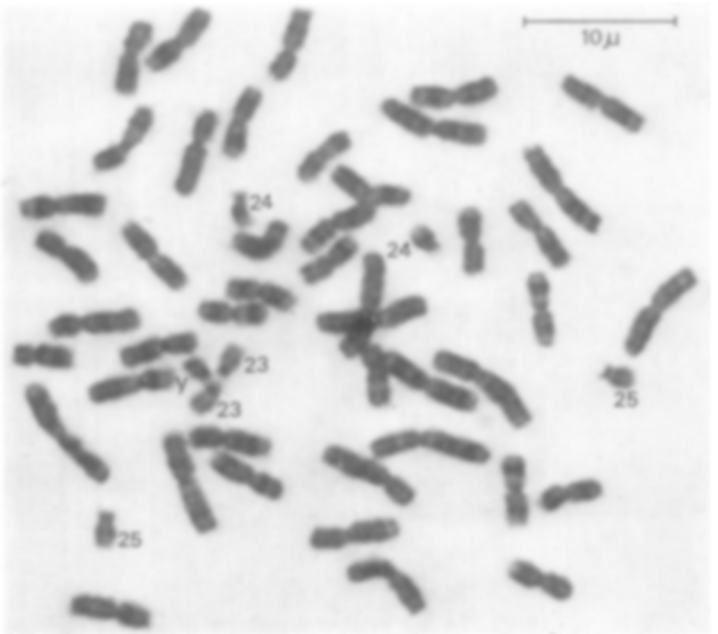


Plate 3. G-banded metaphase spread of *Hylobates* (*Nomascus*) *concolor* (male), showing the banding patterns of the six acrocentric elements and the Y chromosome. Note the participation of the Y chromosome in a chromosome association group.



of the single pair of acrocentrics (pair 24) sometimes, especially in spreads with short condensed chromosomes, bear minute satellites attached to the short arm portion. In less condensed plates, however, these satellites are seldom seen, and the acrocentrics appear to possess a terminal secondary constriction. Typical end-to-end associations, with these terminal secondary constrictions close together or even overlapping, are often observed (Plate 2, a, b, c). These associations resemble those of man (Zang & Back, 1968; van Hemel, 1971), the anthropoid apes (Ardito *et al.*, 1973; Miller *et al.*, 1974) and a number of other primates (de Boer, 1973*a*, 1973*b*, 1974).

In *Hylobates* (*Nomascus*) *concolor* (Plate 1, c) 44 of the autosomes are biarmed, and like in *Symphalangus syndactylus* most of them are nearly metacentric and can hardly be individualized. The X chromosome is one of the longest metacentrics; the Y is a small acrocentric element. Six autosomes (pairs 23, 24 and 25) are acrocentric, all of approximately the same size as the Y chromosome. None of them bears clear satellites, but at least several (the number varies from plate to plate) show small tufts of uncondensed chromosome material attached to the short arm portions. End-to-end associations of these small acrocentrics are found in nearly all metaphase spreads. In spreads of the male as well as the female specimens one group of two, three or four acrocentrics (Plate 2, g–j), and sometimes two groups of two acrocentrics (Plate 2, k) are seen in association. A group of four associating acrocentrics resembles a crosswise association of the marked chromosomes of the subgenus *Hylobates*. In the female specimens never more than four elements in the same cell are involved in such associations. In the male specimen, however, also cells occur with a group of two and a group of three associating acrocentrics (Plate 2, l). Since the G-banding pattern allows individualization of the three pairs of small acrocentrics and the Y chromosome (see Plate 3), it was possible to trace which elements exactly participate in these associations. It was found, that though never more than four autosomes are involved in associations in the same cell, the members of each of the three pairs of small acrocentrics can participate. In the male specimen also the Y chromosome sometimes is involved in the associations (Plate 3), which probably accounts for the incidental finding of five associating acrocentrics in one spread.

This participation of the Y chromosome of *Nomascus* in associations is remarkable, since neither in man nor in the anthropoid apes, in which more or less comparable associations of several pairs of satellited acrocentrics are found (Zang & Back, 1968; Ardito *et al.*, 1973; Miller *et al.*, 1974), the Y chromosome associates. However, in man some cases are known of a translocation of the satellite part of an acrocentric autosome to the Y chromosome, with the result that the Y becomes also involved in the associations (Genest, 1972). In a similar way the associating Y chromosome of *Nomascus* might have originated. That, however, would mean that male individuals possess more heterochromatic material (located in the secondary constriction region of the small acrocentrics) than the females; in other words, that males are heterozygous for a duplication of at least part of the short arm of one of the acrocentric autosomes. In itself, the difference in the number of elements capable of associating in males and females does not seem to be very important, since in *Nomascus* like in man and the anthropoid apes never all of the elements capable of associating are actually found in association in the same cell. Moreover, in man many cases are known of duplications of the short arm of associating acrocentrics (Luciani *et al.*, 1968; Al-Aish, 1970), and generally these short arms are fairly variable in man (de la Torre & Giménez-Martin, 1972; Geraerdts & Pearson, 1974) as well as in the chimpanzee and the gorilla (Pearson, 1973; Dutrillaux *et al.*, 1973; Lin *et al.*, 1973;

Miller *et al.*, 1974). Therefore, it seems that an excess of the amount of (at least part of) this heterochromatic material does not lead to abnormal features; rather a certain minimum seems to be necessary (see also Geraerdts & Pearson, 1974).

Using a technique specifically staining "nucleolus organizing" chromosome regions (N-banding technique), Matsui & Sasaki (1973) and Funaki *et al.* (1975) have shown that these regions in man are located in the short arms of the acrocentric chromosomes, while in *Macaca fascicularis* this region is exclusively found in the large secondary constrictions of the marked chromosomes. Therefore, it seems quite clear now that the secondary constrictions of the acrocentric chromosomes of man and the anthropoid apes and the large secondary constrictions of the marked chromosomes of Cercopithecidae, *Hylobates*, *Symphalangus* and many other primates do not only share the typical association behavior in the metaphase, but also have comparable functions in the cell cycle.

Like the short arms of the human and ape acrocentrics, also the marked chromosomes of other primates are quite variable. Often large differences are found in the length of the secondary constriction in different animals and even of both homologues in one metaphase spread (see Plate 1, a), and in at least one case (de Boer, 1971) the occurrence of a duplication in the secondary constriction region seemed very likely. Moreover, in long term tissue cultures the marked chromosomes seem to be more susceptible to changes (pericentric inversion, translocation) than the other chromosomes (Egozcue, 1971; Paztor & Hu, 1973). Phylogenetically seen, the marked chromosomes also seem to be subject to many changes. In the Galagidae for instance, these chromosomes may be either acrocentric (like in *Cercopithecus*), metacentric (like in *Hylobates*) or acrocentric with a terminal secondary constriction (like in *Symphalangus*) (de Boer, 1973*a*), while in *Cercopithecus* in which they are usually acrocentric, a single species (*C. nigroviridis*) occurs in which they are of the same type as in the subgenus *Hylobates* (Ardito & Mortelmans, 1975).

Thus, in the Hylobatidae the occurrence of a pericentric inversion may easily explain the difference between the marked chromosomes of the subgenus *Hylobates* and the associating acrocentrics of *Symphalangus*. On the exact relation between the associating elements of *Nomascus* and those of the other hylobatids however, can only be speculated so far (see Chiarelli, 1973), while also the relation to the association system in man and the anthropoid apes (in which in man as many as five chromosome pairs are involved) is as yet unclear. Bearing in mind the obvious karyological differences within the Hylobatidae (especially those concerning the associating chromosomes, the diploid numbers and the structures of the Y chromosomes) and the fact that marked chromosomes easily change during evolution, it seems advisable to be very careful in linking the Hylobatidae with the Colobinae (Cercopithecidae) because of the occurrence of similar marked chromosomes and identical chromosome numbers (44) in both groups. The *Hylobates* metacentric marked chromosomes could very well have originated independently, while the exact extend of the karyological similarities between the complements with a diploid number of 44 of both groups remains still to be traced by detailed comparisons of banding patterns.

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