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MOLECULAR CYTOGENETIC ANALYSIS OF THAI SOUTHERN PIG-TAILED MACAQUE (*MACACA NEMESTRINA*) BY MULTICOLOR BANDING

X. FAN [1], W. SANGPAKDEE[2], A. TANOMTONG [2], A. CHAVEERACH [2], K. PINTHONG [1,2,3], S. PORNNARONG [1,2], W. SUPIWONG [1,2], V. A. TRIFONOV[1,4], G. G. HOVHANNISYAN [5], R. M. AROUTIOUNIAN [*5], T. LIEHR [**1], A. WEISE [1]

[1] *Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Germany*
[2] *Department of Biology Faculty of Science, Khon Kaen University, Thailand*
[3] *Faculty of Science and Technology, Surindra Rajabhat University, Thailand*
[4] *Institute of Molecular and Cellular Biology, Russia*
[5] *Chair of Genetics and Cytology YSU, Armenia*

In previous studies origin of human and ape chromosomes have been analyzed by comparative chromosome banding analysis and/or by fluorescence *in situ* hybridization (FISH). In the present study FISH-banding, i.e. multicolor banding using probes derived from *Homo sapiens* was applied to reanalyze the chromosomes of Thai southern pig-tailed macaque (*Macaca nemestrina*). The results agree with those of previous studies in other macaques, e.g. *Macaca sylvanus*. Thus, genetic differences leading to the observed large morphological differences within the *Ceropithecoidae* must be in the subchromosomal or even epigenetic level and have to be discovered yet.

***Keywords*:** FISH analysis, pig-tailed macaque, multicolor banding.

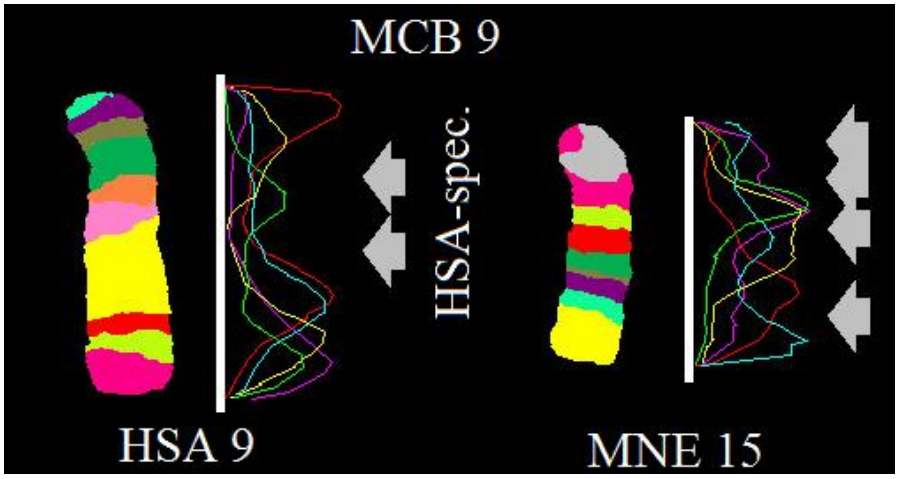
Introduction. Since the early 1980s the idea is pursued that cytogenetic studies in ape species could provide substantial contributions to better understandding of evolutionary history of primate and human phylogeny [1, 2]. After introduction of molecular cytogenetics, especially multicolor-fluorescence *in situ* hybridization (FISH), FISH studies using whole chromosome painting probes and FISH-banding approaches [3] were yet applied. However, the latter was not used systematically to study the question of karyotype evolution in primates; only occasionally studies were performed [4–6]. The most common applied FISH-banding approach is the so-called multicolor banding (MCB), which in contrast to other FISH-banding techniques is anchored in the human DNA-sequence [7]. MCB was already successfully applied for characterization and comparative molecular cytogenetic mapping of the following primate species before: *Gorilla gorilla* [8],

[*] E-mail: genetik@ysu.am [**] E-mail: Thomas.Liehr@med.uni-jena.de

Hylobates lar [9], *Trachypithecus cristatus* and *Macaca sylvanus* (MSN) [10]. Recently, next generation sequencing was also introduced to answer the question: what are the differences between the species. Interestingly, basic cytogenetic data are needed essentially for exact alignment of these new complex datasets [11].

Here we present the first MCB-based study for characterization of the Thai southern pig-tailed macaque (*Macaca nemestrina,* MNE). Macaques (*Catarrhini*, *Ceropithecoidae*, *Cercopithecinae*, *Papionini*) underwent a radiation in Pliocene or Pleistocene, i.e. during the last 3–5 million years [12]. Macaques can be found from Western Africa to far Eastern Asia presenting in many morphologically diverse species. Surprisingly, on the chromosomal level this group is kept absolutely constant: 42 chromosomes with no differences on the cytogenetic level [13]. This is also underlined by the fact that different macaque species can form hybrids, even fertile ones, easily [14].

MSN was studied before by banding cytogenetics [13] and FISH using a low resolution FISH-banding approach [5]. Here we provide the first MCB-based FISH-banding study in MSN.



Representative example for MCB-result obtained in MNE. Result of MCB 9 probe set applied on a human chromosome 9 (HSA) compared to the result obtained on homologous MNE chromosome 15. For each chromosome pseudocolor depiction and underlying fluorochrome profiles are shown. The human chromosome has HSA specific amplification in 9q12 (arrows). The MNE chromosome 15 has compared to HAS insertion of an unknown DNA in 9q34 (gray) and several rearrangements (arrows) – see Table.

Material and Methods. Peripheral blood of MSN (1 male) was acquired in Thailand. Blood lymphocytes from heparinzed blood were subjected to short term culture and cytogenetic work-up using standard procedures.

24 MCB probes derived from human chromosomes *Homo sapiens* (HSA) were applied in 24 independent FISH-experiments in MSN-chromosome-preparations as previously was reported [8]. Evolutionary conserved chromosomal breakpoints were characterized with respect to the human chromosome complement (see Table).

Results and Discussion. In comparison with human karyotype the present MCB study was revealed in MSN 43 evolutionary break-events (see Table). The nomenclature of macaque chromosomes used here was adapted from [2]. As there

are different nomenclatures around these points have to be stressed – e.g. MSN chromosomes 12 and 13 have the designations 9 and 15 elsewhere [15]. Here reported breakpoints were identical to those known from other macaque species, living in Western Africa, while MSN is situated thousands of kilometers away from Thailand [10, 16] (see Table).

Less surprisingly HSA amplifications present in 1q12, 9q12, 16q11.2 and Yq12 were not present in MSN of the corresponding regions (see Fig.). On the other hand, species of specific amplifications of unknown genetic material was present in MSN in regions homologous to HSA 9q34, 17p10 and 17q24. For the latter 17q24 complex regions of segmental duplication were reported [17]. Also species of specific amplifications are suggested to play a major role in speciation [8].

Breakpoints of MNE according to MCB (Abbreviations: cen = centromeric position)

MSN	MSN chromosomes given as derivatives of human chromosomes	cen
1	inv(1) (q23.3q42.13), dim(1) (q12)	1q42.13
2	der(3) (qter – > q27.3::p22.3 – > p24::q22.1 – > q27.3::p22.3 – > – > p12.3::p26.3 – > p24::q22.1 – > p12.3:)	3q26.1
3	der(7) (21qter – > 21q11.2::7p22.3 – > 7p22.1::7q21.3 – > – > 7q22.1::7q11.23 – > 7p21.3::7p21.3 – > 7q11.23::7q22.1 – > 7qter)	like HSA 7
4	inv(6) (p24q25.2) and inv(6) (q21q25.2)	6q24.3
5	inv(4) (p15.3q10)	like HSA 4
6	no change to HSA 5	like HSA 5
7	der(15)t(14; 15) (q11.2; q26.3)	15q25
8	no change to HSA 8	like HSA 8
9	inv(10) (q11.23q22.3)	like HSA 10
10	der(20) (22qter – > 22p13::20p11.21 – > 20p13::20q11.21 – > 20qter)	like HSA 22
11	no change to HSA 12	like HSA 12
12	inv(2) (q14.1q21.1)	2q22.1
13	inv(2) (q11.1q14.1)	2p11.2
14	inv(11) (p15.4q13.4)	11p15.4
15	der(9) (9qter – > 9q34::?::9q34 – > 9p24.3::9q21.11 – > 9q22.33:), dim(9)(q12)	9q33.2
16	der(17) (pter – > p10::?::p10 – > q12::q23.3 – > q21.32::q12 – > – > q21.32::q23.3 – > q24::?::q24 – > qter)	like HSA 17
17	no change to HSA 13	13q21.31
18	no change to HSA 18	18q21.2
19	no change to HSA 19	like HSA 19
20	inv(16) (q22.1q22.3), dim(16) (q11.2)	like HSA 16
X	no change to HSA X	like HSA X
Y	del(Y) (q12q12)	like HSA Y

Centromeric regions in MSN 3, 5, 6, 8–11, 16, 19, 20, X and Y were identical to human centromeric positions (see Table). As the centromeric regions, even if being intraspecifically stable, do not contain identical alphoid DNA stretches [18] it is suggested that these chromosomal region evolve faster than other genomic regions. Centromere repositioning as discussed elsewhere [19] was observed for MSN 1, 2, 4, 7, 12–15, 17 and 18 (see Table).

Conclusion. Recently we studied *Macaca sylvanus* (MSY) [10] by MCB. Interestingly, neither at cytogenetic, nor at FISH-banding level differences between MSY and MNE were found. Altogether, the present study is confirmed in details for another macaque species and the general chromosomal composition cannot be the reason for specification in this genus.

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