A Discovery of Polymorphism of Nucleolar Organizer Regions (NORs) and Whole-Arm Translocation (WAT) between Chromosome 8 and 9 of Lowland Agile Gibbon (Hylobates agilis unko) in Thailand

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Received October 17, 2009; accepted January 10, 2010

Summary  This paper succeeded in discovering the polymorphism of nucleolar organizer regions (NORs) and whole-arm translocation (WAT) between chromosomes 8 and 9 of the lowland agile gibbon (Hylobates agilis unko) in Thailand, after standard whole blood lymphocyte culture and G-banding technique were applied to stain the chromosomes. The results showed that the 2n of H. agilis unko was 44. The type of autosomes were 28 metacentric, 12 submetacentric and 2 acrocentric chromosomes, with the X and Y-chromosome being submetacentric and acrocentric chromosome, respectively. In addition, a pair of the long arms of chromosome 12 showed clearly observable NORs. This is the first report on the polymorphism of NORs in H. agilis unko. The results show a heteromorphism in female with a difference size of NORs of chromosome 12, while in males show an equal size of both chromosome 12 with a homomorphism. We also detected a WAT between chromosomes 8 and 9 in H. agilis unko and found that both male and female chromosomes 8 and 9 were 8c/H11032 (homomorphism, 8c/H11032 8c/H11032) and 9/H11032 (homomorphism, 9/H11032 9/H11032), respectively, resulting from pericentric inversion and followed by reciprocal translocation.

Key words Polymorphism, Nucleolar organizer regions (NORs), Whole-arm translocation, Lowland agile gibbon (Hylobates agilis unko).

The family Hylobatidae (small apes) are included in Appendix I (threatened species) of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) as endangered species, together with great apes and other monkeys (Soehartono and Mardiastuti 2002). Gibbons (genus Hylobates) constitute a sister group to the great apes (Pongidae) and humans (Hominidae) (Groves 1972). The 4 genera of gibbons recognized by classical taxonomic criteria are further characterized by 4 unique karyotypes and diploid numbers (Chiarelli 1972, Markvong 1973, Prouty et al. 1983). Gibbons show a variable chromosome number ranging from 2n (diploid)=38 to 2n=52, which provides a useful landmark to identify the genera of this family, Hylobates 2n=44, Hoolock 2n=38, Nomascus 2n=52 and Symphalangus 2n=50 (Prouty et al. 1983, Marshall and Sugardjito 1986, Geissmann 1995).

Comparative cytogenetic studies reveal striking degrees of genomic rearrangement since divergence of the 4 groups from a common ancestor (Dutrillaux et al. 1975, Myers and Shafer 1979, Couturier et al. 1982, Prouty et al. 1983, Van Tuinen and Ledbetter 1983). However, it was
proposed in a molecular analysis that these groups should be classified as 4 genera because the molecular distances between the 4 gibbon genera were in the same range as those between *Homo* and *Pan*, or even higher (Roos and Geissmann 2001). On the other hand, the gibbon has a very differentiated karyotype from humans (Jauch *et al.* 1992, Koehler *et al.* 1995, Nie *et al.* 2001, Hirai *et al.* 2003).

Virtually all modes of chromosomal rearrangement observed in karyotypic divergence of mammals are recognized in gibbons; pericentric and paracentric inversion, Robertsonian translocation and tandem fusion, chromosomal fission and reciprocal translocation (Dutrillaux *et al.* 1975, Couturier *et al.* 1982, Van Tuinen and Leadbetter 1983). Indeed, among the earliest studies conducted, pericentric inversion heteromorphism was noted in Mueller’s gibbon (*H. muelleri*), one of the 6 species of the genus *Hylobates* that all possess 2n=44 (Tantravahi *et al.* 1975). Inversion heteromorphism for the same chromosome pair was noted in one of two *H. agilis* (Van Tuinen and Ledbetter 1983). A more comprehensive study of the 6 *Hylobates* species (Stanyon *et al.* 1987) revealed the existence of 3 distinct pericentric inversion morphs involving the same chromosomal pair, with *H. muelleri*, moloch gibbon (*H. moloch*) and *H. agilis* maintaining all 3 morphs.

Fig. 1. Metaphase chromosome plates with homomorphism chromosomes 12 in size of nucleolar organizer regions (NORs) and homomorphism chromosomes 8 and 9 (8c’8c’ and 9’9’) of whole-arm translocation (WAT) or reciprocal translocation of male lowland agile gibbon (*Hylobates agilis unko*) in Thailand, 2n=44 by G-banding technique. Arrows indicate the labeled NORs, chromosomes 8, 9 and sex-chromosomes.
The nature of the contrasts between the 4 karyotypes in gibbons led to the hypothesis that reciprocal translocation predominated in the chromosomal evolution of gibbons (Van Tuinen and Ledbetter 1983, Van Tuinen et al. 1999). While the magnitude of genomic restructuring has rendered the interpretation of many translocation events difficult or impossible, this hypothesis was corroborated by gene mapping and in situ hybridization, which revealed novel gene linkage groups most consistent with translocation (Turleau et al. 1983, Cochet et al. 1987, Van Tuinen and Ledbetter 1983, Arnold et al. 1996, Van Tuinen et al. 1999). In the present study, we investigated a complex cytogenetic polymorphism that is possibly an intermediate stage in the differentiation of the karyotype of H. agilis unko.

Materials and methods

Blood samples were taken from 2 males and 1 female H. agilis unko, which were kept in Songkla Zoo, Thailand. T-lymphocytes were cultured by the whole blood microculture technique and G-banding technique modified from Rooney (2001) and Kampiranont (2003).
Results and discussion

The results showed that the $2n$ of *H. agilis unko* was 44, a result which agrees with Chiarelli (1972), Stanyon *et al.* (1987), Van Tuinen *et al.* (1999), Hirai *et al.* (2003, 2005). The type of autosomes were 28 metacentric, 12 submetacentric and 2 acrocentric chromosomes, with the X and Y-chromosomes being submetacentric and acrocentric chromosome, respectively (Fig. 3).

In addition, a pair of the long arms of chromosome 12 showed clearly observable NORs. This is the first report on polymorphism of NORs in *H. agilis unko* in Thailand, $2n=44$ by G-banding technique. Arrows indicate the labeled NORs, chromosomes 8, 9 and sex-chromosomes.

Fig. 3. Metaphase chromosome plate and karyotype with homomorphism chromosomes 12, homomorphism chromosomes 8, 9 ($8c'8c'$ and $9'9'$) of male (A) and a new discovery polymorphism chromosomes 12, homomorphism chromosomes 8, 9 ($8c'8c'$ and $9'9'$) of female (B) lowland agile gibbon (*Hylobates agilis unko*) in Thailand, $2n=44$ by G-banding technique. Arrows indicate the labeled NORs, chromosomes 8, 9 and sex-chromosomes.

A. The male lowland agile gibbon

B. The female lowland agile gibbon

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In addition, a pair of the long arms of chromosome 12 showed clearly observable NORs. This is the first report on polymorphism of NORs in *H. agilis unko*. The results show heteromorphism in the female with a difference size of NORs of chromosome 12, while in males an equal size of both chromosome 12 with homomorphism (Figs. 1, 2 and 5) was shown, that consistent to the report of
Warburton et al. (1975) found a difference in size of NORs (polymorphism) in white-handed gibbons (*H. lar*), while Van Tuinen et al. (1999) found the same polymorphic NORs in *H. moloch*.

Nucleolar organizer regions are composed of high amounts of rDNA, protein, and RNA. Ribosomal DNA is functioning on 28S and 18S rRNA synthesis in mammals. The rDNA, repetitive DNA, functions in rRNA synthesis so the increase or decrease in the size of NORs in *H. agilis unko* might be influential to the gene in protein synthesis (Kampiranont 2003). Warburton et al. (1975) found polymorphism in macaques (genus *Macaca*). However, Tantravahi et al. (1976) and Miller (1977) reported that there are NORs on human chromosomes 13, 14, 15, 21, 22 and also found that human satellite chromosomes have polymorphism by NOR-banding technique.

We also detected a WAT between chromosomes 8 and 9 in *H. agilis unko* and found that both male and female chromosomes 8 and 9 were 8c’ (homomorphism, 8c’8c’) and 9’ (homomorphism, 9’9’), respectively, resulting from pericentric inversion and followed by reciprocal translocation (Figs. 1–4). The result is consistent with the report of Hirai et al. (2005) that revealed polymorphism in *H. agilis unko* and highland agile gibbons (*H. agilis agilis*) with 3 forms (8a, 8b, 8c’) of chromosomes 8 while there are 2 forms (9, 9’) of chromosomes 9, however there are 3 forms (8a, 8b, 8c) and only 1 form (9) in Bornean agile gibbons (*H. agilis albibarbis*) chromosomes 8 and 9, respectively. Furthermore, our discovery is similar to the report of Van Tuinen et al. (1999) that indicated 3 forms (8a, 8b, 8c’) and 2 forms (9, 9’) polymorphisms of *H. agilis unko*, respectively. However, there are 2 forms (8a, 8c’) and 2 forms (9, 9’) of polymorphism in both of *H. agilis agilis* and *H. agilis albibarbis*. Our result contrasts that of Stanyon et al. (1987) whose report indicated 3 forms (8a, 8b, 8c) and only 1 form (9) polymorphisms in *H. agilis*.

There are various studies of WAT between chromosomes 8 and 9 in other gibbon species (*2n=44, genus Hylobates*). Stanyon et
al. (1987) reported the existence of 2 forms (8b, 8c) and only 1 form (9) of polymorphism in H. lar, there are the forms of 8b and 9 polymorphisms in pileated gibbon (H. pileatus), while H. molochn and Mueller’s gibbon (H. muelleri) have 3 forms (8a, 8b, 8c) and 1 form (9) of polymorphism. The report of Van Tuinen et al. (1999) showed that all of H. lar, H. muelleri, H. molochn and H. pileatus have only 1 form (9) of chromosome 9 while there are 3 forms (8a, 8b, 8c), 3 forms (8a, 8b, 8c), 2 forms (8b, 8c) and only 1 form (8b) of H. lar, H. muelleri, H. molochn and H. pileatus chromosome 8, respectively. Moreover, there are 2 forms (8a, 8c’), 2 forms (8a, 8c) and 2 forms (8a, 8b) of chromosome 8 of H. muelleri funereus×H. agilis agilis, H. muelleri funereus×H. agilis albifurcatus and H. lar×H. agilis agilis, respectively. While there are the existence of 2 (9, 9’), 1 (9) and 1 (9) forms of the above 3 hybrid gibbons chromosome 9, respectively. In addition, Hirai et al. (2005) revealed that H. meulleri chromosomes 8 and 9 have 3 forms (8a, 8b, 8c) and only 1 form (9) of polymorphism.

There are 5 forms of chromosome 8 (8a, 8b, 8c, 8c’ and 9’) of H. agilis. Chromosome 8a can be derived from 8b by a single pericentric inversion while chromosome 8c derived from 8b requires at least 2 pericentric inversions. Morphs 8c is then involved in a reciprocal translocation with chromosome 9. Artificial products are shown alongside actual morphs 8c’ and 9’ (Hirai et al. 2003, 2005) (Fig. 4). This is the first report on WAT between chromosome 8 and 9 of H. agilis unko in Thailand. The gibbon samples used (2 males and 1 female) are too few in number for detection of polymorphism in general primates but the problem is a result of the limited amount of H. agilis unko in The Zoological Park Organization Under the Royal Patronage of H.M. The King.

The report of Van Tuinien et al. (1999) revealed the study of meiosis cell division of 2 male H. agilis with an inversion-translocation heterozygote (8a8c’) by using testicular biopsy, C-banding and electron-microscope analysis. The results showed that there are 20 bivalents (2 centromeres) and a single ring quadrivalent (4 centromeres) in diakinesis (metaphase I). The single ring quadrivalent results from the synapsis of reciprocal translocation homologous chromosomes 8 and 9. Furthermore, the results described that direct evidence for fertility is the production of normal offspring by the compound heterozygotes.

Our studies demonstrate that gibbons have more rapid karyotypic change than other primates, consistent with Jones (1994) who reported on karyotypic change in primates that can be divided into 3 levels as follow: slow, including tribe Papionini and family Callitrichidae; medium, including family Pongidae, Hominidae, most Cebidae except subfamily Aotinae and tribe Cercopithecini; and fast, including family Hylobatidae, subfamily Aotinae and tribe Cercopithecini.

Acknowledgements

The financial support from The Zoological Park Organization Under the Royal Patronage of H.M. The King is gratefully acknowledged. We also thank Mr. Sopon Dumnui, Director of the Organization, and Dr. Sumat Kamolnaranath, Chief of the Educational Division and Director of Songkla Zoo, for the valuable help.

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