A Comparison of the Karyotype of Five Species in Genus *Macaca* (Primate, Cercopithecidae) in Thailand by Using Conventional Staining, G-banding and High-Resolution Technique

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Summary Cytogenetics of 5 macaque species from genus *Macaca* in Thailand were studied using lymphocyte cultures and high-resolution techniques. Their chromosome numbers are $2n=42$, 20 pairs of autosome, and 1 pair of sex-chromosome. *M. arctoides* and *M. mulatta* have a fundamental number (NF) 84 in male and female but the others have 83 in male and 84 in female. They have 6 large, 4 medium, 8 small metacentric chromosomes and 8 large, 12 medium, 2 small submetacentric chromosomes respectively. *M. fascicularis* and *M. mulatta* have medium metacentric X chromosome. *M. assamensis*, *M. nemestrina* and *M. arctoides* have medium submetacentric X chromosome. *M. arctoides* has small submetacentric Y chromosomes, *M. mulatta* has small metacentric Y chromosomes, while *M. assamensis*, *M. fascicularis* and *M. nemestrina* have small telocentric Y chromosomes. By using G-banding in metaphase and high-resolution technique in late prophase, the results show that the bands are 274, 273, 273, 275, 273 and 351, 350, 350, 352, 350 respectively. Their autosome and X chromosome are similar but their Y chromosome is different. The NOR is on the short arm of chromosome 13. The largest is the metacentric chromosome. The smallest is the Y chromosome. Thus X and Y chromosomes can use for identification.

Key words Macaque, *Macaca*, Karyotype, G-banding, High-resolution technique.

Over the world, primates are separated into 13 families, 60 genera and 232 species. Three families, 5 genera and 13 species are found in Thailand (Wilson and Cole 2000). Five species are the macaques in genus *Macaca*. These are stump-tailed macaque (*Macaca arctoides*: Geoffroy 1831), assam macaque (*M. assamensis*: McClelland 1839), long-tailed macaque (*M. fascicularis*: Raffles 1821), rhesus monkey (*M. mulatta*: Zimmermann 1780) and pigtailed macaque (*M. nemestrina*: Linnaeus 1766) (Brockelman 2524, Lekagul and McNeely 1977, 1988).

Although cytogenetics of the macaques in genus *Macaca* have been reported (Chiarelli 1962, Hsu and Benirschke 1967, Napier and Napier 1976, Caballin *et al.* 1980, Small and Stanyon 1985, Brown *et al.* 1986 and Hirai *et al.* 1991), there are few reports of the cytogenetics of the macaques in this genus in Thailand. The macaques in Thailand are conserved animals. They are at risk. In this study, lymphocyte culture, conventional staining, G-banding and the high-resolution technique were used to compare the results with the previous studies. In the future, basic knowledge about macaques and their cytogenetics will be applied for research and to protect them from extinction.

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Materials and methods

M. arctoides, *M. assamensis*, *M. fascicularis*, *M. mulatta* and *M. nemestrina* were maintained in Nakhonratchasima Zoo, Nakhonratchasima Province and Songkla Zoo, Songkla Province, Thailand. Blood samples from 2 male and 2 female macaques were collected. T-lymphocytes were cultured by the whole blood microculture method. Conventional staining, G-banding and high-resolution technique modified from human (Kumpiranon 1994) were used.

Results

The chromosome numbers of 5 species of *Macaca* in Thailand are similar, $2n=42$. They are composed of 40 autosomes (20 pairs) and 2 sex chromosomes (X and Y chromosome), 1 pair. The results show that all of them have the same fundamental number (NF) in females. *M. arctoides*, *M. mulatta*, *M. assamensis*, *M. fascicularis* and *M. nemestrina* have 84 in female. However the fundamental numbers in male are different. *M. arctoides* and *M. mulatta* have 84 but *M. assamensis*, *M. fascicularis* and *M. nemestrina* have 83 (Table 1).

All Thai macaques have only 2 types of autosome, metacentric and submetacentric. They have 6 large, 4 medium and 8 small metacentric chromosomes and 8 large, 12 medium and 2 small submetacentric chromosomes (Table 1).

X chromosomes of *M. fascicularis* and *M. mulatta* are medium metacentric but *M. assamensis*, *M. nemestrina* and *M. arctoides* are medium submetacentric. Y chromosomes of *M. arctoides* are small submetacentric chromosomes, while *M. mulatta* has small metacentric chromosomes. *M. assamensis*, *M. fascicularis* and *M. nemestrina* have small telocentric chromosomes (Table 1).

The genetic marker is the unique chromosome of the living thing. The results suggest that the genetic marker of these macaques is chromosome 13. This is because this chromosome has a secondary constriction and nucleolar organizer region (NOR) on the short arm. The metacentric autosome is the largest chromosome. The Y chromosome is the smallest.

G-banding was used in the metaphase and high-resolution technique was used in the late prophase. The results show that the bands of autosome and sex chromosome are haploid set. The

Table 1. Diploid ($2n$) and fundamental number (NF) of autosome and sex chromosome of 5 species of macaques in Thailand

Type of macaques	NF	$2n$	Autosome (pairs)						Sex chromosome	
			Metacentric			Submetacentric			X	Y
			L	M	S	L	M	S		
Stump-tailed macaque (<i>Macaca arctoides</i>)	84 in male and female	42	3	2	4	4	6	1	sm (M)	sm (S)
Assam macaque (<i>M. assamensis</i>)	83 in male 84 in female	42	3	2	4	4	6	1	sm (M)	t (S)
Long-tailed macaque (<i>M. fascicularis</i>)	83 in male 84 in female	42	3	2	4	4	6	1	m (M)	t (S)
Rhesus monkey (<i>M. mulatta</i>)	84 in male and female	42	3	2	4	4	6	1	m (M)	m (S)
Pigtailed macaque (<i>M. nemestrina</i>)	83 in male 84 in female	42	3	2	4	4	6	1	sm (M)	t (S)

Note: m=metacentric; sm=submetacentric; t=telocentric; L=large chromosome; M=medium chromosome; S=small chromosome.

Fig. 1. Karyotype of male (A) and female (B) stump-tailed macaque (*M. arctoides*) $2n$ (diploid)=42, male (C) and female (D) assam macaque (*M. assamensis*) $2n$ (diploid)=42 using conventional staining.

Fig. 2. Karyotype of male (A) and female (B) long-tailed macaque (*M. fascicularis*) $2n$ (diploid)=42, male (C) and female (D) rhesus monkey (*M. mulatta*) $2n$ (diploid)=42 using conventional staining.

bands in the metaphase are 274, 273, 273, 275 and 273 respectively. The bands in the late prophase are 351, 350, 350, 352 and 350 respectively. The bands of autosome and X chromosome from *M. arctoides*, *M. assamensis*, *M. fascicularis*, *M. mulatta* and *M. nemestrina* are similar but the difference occurs in the Y chromosome.

Discussion

From this study it was found that the chromosome number and the fundamental number of 5 species of *Macaca* in Thailand are consistent with Brown *et al.* (1986). The *Macaca*, namely *M. arctoides*, *M. fascicularis*, *M. mulatta*, *M. nemestrina*, Celebes crested, Bonnet and Mangabey in genus *Cercocebus* have the same chromosome number, $2n=42$. This research agrees with other previous studies. Hirai *et al.* (1991) reported that the chromosome numbers of *M. arctoides* is $2n=42$ and the NF is 83 in male and female. Napier and Napier (1976), Small and Stanyon (1985) found that the chromosome number of *M. mulatta* is $2n=42$.

In this study, the autosomes were different from Brown *et al.* (1986) who reported that *M. arctoides* has 14 metacentric and 26 submetacentric chromosomes whereas the chromosomes of *M. fascicularis*, *M. mulatta* and *M. nemestrina* were similar, with 18 metacentric and 22 submetacentric. *M. fascicularis* (Hirai *et al.* 1991) and *M. mulatta* (Napier and Napier 1976, Small and Stanyon 1985) are also reported as having 18 metacentric and 22 submetacentric chromosomes.

The results for X and Y chromosomes from this research agree with previous reports that indicate that X chromosomes of *M. arctoides*, *M. fascicularis*, *M. mulatta* and *M. nemestrina* are submetacentric, metacentric, metacentric and submetacentric respectively and the Y chromosome is the

Fig. 3. Karyotype of male (A) and female (B) pigtailed macaque (*M. nemestrina*) $2n$ (diploid)=42 using conventional staining.

Fig. 4. Karyotype of male (A) and female (B) stump-tailed macaque (*M. arctoides*) $2n$ (diploid)=42, male (C) and female (D) assam macaque (*M. assamensis*) $2n$ (diploid)=42 using G-banding.

Fig. 5. Karyotype of male (A) and female (B) long-tailed macaque (*M. fascicularis*) $2n$ (diploid)=42, male (C) and female (D) rhesus monkey (*M. mulatta*) $2n$ (diploid)=42 using G-banding.

Fig. 6. Karyotype of male (A) and female (B) pigtailed macaque (*M. nemestrina*) $2n$ (diploid)=42 using G-banding.

smallest (Brown *et al.* 1986). The X chromosome of *M. fascicularis* is medium metacentric and the Y chromosome is the smallest telocentric reported (Hirai *et al.* 1991). *M. assamensis* has medium submetacentric X chromosome and the smallest is the Y chromosome (Napier and Napier 1976, Small and Stanyon 1985).

The sex chromosomes of the macaque in genus *Macaca* has a large shift. Their X and Y chromosomes have different shape and size. The Y chromosome has three types, metacentric, submetacentric and telocentric. The X chromosome has 2 types, metacentric and submetacentric. According to a previous study it was found that *M. arctoides*, *M. fascicularis*, *M. mulatta*, *M. nemestrina*,

Fig. 7. Karyotype of male (A) and female (B) stump-tailed macaque (*M. arcoides*) $2n$ (diploid)=42, male (C) and female (D) assam macaque (*M. assamensis*) $2n$ (diploid)=42 using high-resolution.

Fig. 8. Karyotype of male (A) and female (B) long-tailed macaque (*M. fascicularis*) $2n$ (diploid)=42, male (C) and female (D) rhesus monkey (*M. mulatta*) $2n$ (diploid)=42 using high-resolution.

Celebes crested and Bonnet have a large shift in sex chromosome (Brown *et al.* 1986). The Y chromosome is the smallest and has different sizes. Perhaps the centromere of the chromosome is not shown and the type of chromosome is not separated (Napier and Napier 1976). *M. mulatta*, *M. nemestrina*, Japanese monkey and Taiwan monkey (*M. cyclopis*) have different size and shape of Y chromosome (Hirai *et al.* 1991). In addition, Stanyon (1983) found the difference of the chromosome of the macaque in genus *Cercocebus* namely the Japanese monkey and *Cercocebus aterrimus* by using C-banding.

The genetic marker of the macaque in genus *Macaca* in Thailand is NOR on the short arm of chromosome 13. This result agrees with the result from Brown *et al.* (1986) that reported *M. arcoides*, *M. fascicularis*, *M. mulatta*, *M. nemestrina*, Celebes crested, Bonnet and Mangabey have NOR on the short arm of chromosome 9. The different position of NOR occurred because of the karyotype and the method used to detect the length of the chromosome. Similarly, NORs of baboon and gibbon are on chromosome 1 (Jones *et al.* 1994). NORs of Japanese monkey and *Cercocebus aterrimus* are on the short arm of chromosome 13 but the size is different (Stanyon 1983). From observation of the metaphase cell of the macaque in genus *Macaca* in Thailand, the first pair of chromosomes is the largest distinctive metacentric, while the Y chromosome is the important chromosome that is used to identify the species of the macaque according to Brown *et al.* (1986), Napier and Napier (1976), Hirai *et al.* (1991) and Small and Stanyon (1985).

The chromosome bands of 5 macaques using G-banding and high-resolution technique, showed low sensitivity when compared with the bands in human and ape that showed 1,000 bands per set of haploid chromosome by using G-banding in prophase.

Using high-resolution banding technique for comparison of the chromosomes in Japanese

monkey and *C. aterrimus*, the differences in their chromosomes are clear using medium detailed band but the number of bands per set of haploid chromosome are not seen (Stanyon 1983). Yunis (1982) used G-banding for studying the evolution of human, gorilla, orangutan and chimpanzee by comparing the characterization and the bands of the chromosome. The metaphase chromosome showed 320–500 bands and the prophase chromosome showed 1,000 bands per set of haploid chromosome. Using G-banding for *M. mulatta* showed more bands in the late prophase chromosome than the metaphase chromosome. This difference is used to couple the chromosomes and easily check mutation in small points (Small and Stanyon 1985).

Fig. 9. Karyotype of male (A) and female (B) pigtailed macaque (*M. nemestrina*) $2n$ (diploid)=42 using high-resolution.

Fig. 10. A comparison of the autosomes between human (left) and macaque (right) show the same (A), similar (B) and different (C) G-banding pattern. And a comparison of sex chromosome show the difference on chromosome Y. (human; Hm, pigtailed macaque; Mn, rhesus monkey; MM, stump-tailed macaque; Mar, long-tailed macaque; Mf and assam macaque; Mas).

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Fig. 11. A comparative idiogram of autosome and sex chromosome between 5 macaque species from genus *Macaca* in Thailand (pigtailed macaque; Mn, rhesus monkey; MM, stump-tailed macaque; Mar, long-tailed macaque; Mf and assam macaque; Mas).

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