

Chapter 3

Genetic Differentiation of Agile Gibbons Between Sumatra and Kalimantan in Indonesia

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Introduction

The gibbons (Hylobatidae) are a diverse group of small apes that have adapted to the rain forests of South and Southeast Asia and radiated into numerous (12–14) discrete species. Recently, the four subgenera of small apes [*Hoolock*, (previously *Bunopithecus*, Mootnick and Groves 2005), *Hylobates*, *Symphalangus*, and *Nomascus*] were all raised to the level of genera because the genetic distances between them indicated by mitochondrial DNA were larger than those between *Homo* and *Pan* (Hayashi et al. 1995; Roos and Geissmann 2001). Some aspects of gibbon classification are still controversial, in particular the differentiation of subspecies (Groves 2001; Brandon-Jones et al. 2004; Mootnick 2006; Chatterjee this volume). Accurate determination of collection localities is critical for diagnosing subspecies in this group, which is morphologically very diverse in some physical features (e.g., pelage pattern, Marshall and Sugardjito 1986; Mootnick 2006). Thus it is important to use animals of known origin for genetic studies, which are crucial for planning the conservation of evolutionarily significant units (ESUs) (Crandall et al. 2000; Frankham et al. 2002). The taxonomy of agile gibbons is disputed (Chatterjee this volume), with some researchers recognizing a single species (*H. agilis*), and others recognizing one species (*H. agilis*) on the Asian mainland and Sumatra and a second species (*H. albibarbis*, or the white-bearded gibbon) on Borneo. In this chapter, we refer to all gibbons in this group as agile gibbons, but identify the Bornean taxon as *H. albibarbis*. We have aimed to collect samples from gibbons of known origin, and consequently have been able to demonstrate cytogenetic and molecular genetic differentiation of agile gibbon taxa between Sumatra and Kalimantan. These results provide important information on their biogeography and ESUs and a basis for future comprehensive evolutionary genetic investigations of small apes. We summarize the essential points of the data obtained so far, and give our point of view in this chapter.

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The genus *Hylobates*, with 44 chromosomes, is known to include inversion polymorphisms in chromosome 8, which are common to several species (Tantravahi et al. 1975; van Tuinen and Ledbetter 1983; Stanyon et al. 1987), a pattern that is rare in chromosome evolution. Apart from this phenomenon, small apes have undergone drastic chromosome changes that can be detected with human chromosome painting probes: all four genera of small apes show dozens of reciprocal translocations that differentiate them from humans and great apes (Jauch et al. 1992; Koehler et al. 1995; Nie et al. 2001; Müller et al. 2003). Accordingly, chromosomes of small apes likely have some special features, as numerous translocations are typically considered disadvantageous.

Most recently, van Tuinen et al. (1999) identified a new translocation between chromosomes 8 and 9 in three taxa (*H. agilis agilis*, *H. agilis unko*, and *H. albibarbis*) of agile gibbons. In concurrent investigations using different samples, Hirai et al. (2003) found the same variation, and based on the combined data from the two studies Hirai et al. (2003) postulated that the translocation may be a chromosome variant specific for Sumatran agile gibbons and absent in the Bornean taxon. To further explore the distribution of this translocation and its significance for gibbon evolution, the Primate Research Institute, Kyoto University, Japan, and the Primate Research Center, Bogor Agricultural University, Indonesia, initiated a joint research entitled “Comprehensive study of subspeciation of agile gibbons.” We started by obtaining genetic samples from animals of known origin and analyzing pelage patterns, chromosome structures, and DNA sequences. Surprisingly, combined analyses of such distinct parameters from the same samples have been very few so far. We were particularly conscious of the need to find gibbons of known origin from captivity, private pet owners, and zoological institutions, because analyses of animals of unknown origin have sometimes produced conflicting results regarding the evolution in the family Hylobatidae. It is very difficult to reliably identify species by their morphology, especially in the genus *Hylobates*, without information on their collection locality.

Samples and Identification

Blood sampling was done with permission from the Indonesian Research Authority/*Lembaga Ilmu Pengetahuan Indonesia* (LIPI) and the Indonesian Ministry of Forestry's Department for the Protection and Conservation of Nature/*Perlindungan Hutan dan Konservasi Alam* (PHKA), and with help from rangers from the local Natural Resources Conservation Offices/*Balai Konservasi Sumber Daya Alam* (BKSDA) in West Sumatra, Central Kalimantan, and South Kalimantan. To obtain samples from as many gibbons of known origin as possible, we conducted interviews with pet owners and zoological institutions about the acquisition process in nearby natural habitats. Chromosome and DNA samples were then imported to Japan following acquisition of the appropriate

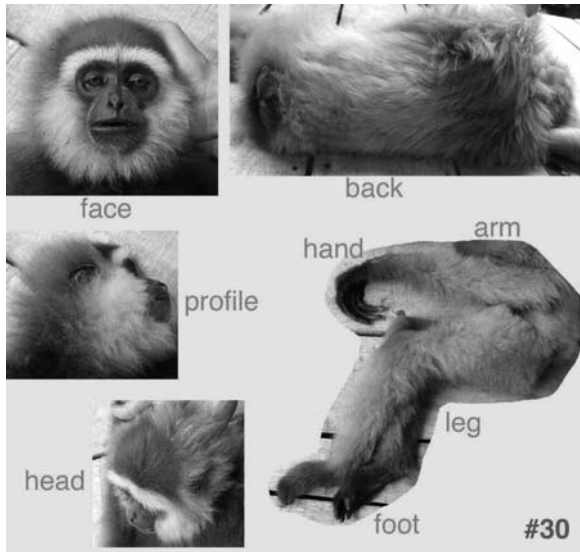


Fig. 3.1 A typical plate of pictures of pelage pattern of *Hylobates agilis albibarbis* from Kalimantan for morphological identification

CITES permits. Species and subspecies identification was conducted with reference to Marshall and Sugardjito (1986), Geissmann (1995), and Mootnick (2006), using information on the locality of collection and pelage photographs of all animals that we collected. We took pictures of the face, profile, back, head, arm, hand, leg, thigh, and foot of each animal while the animal was under ketamine anesthesia (e.g., Fig. 3.1). Pictures of the pelage of all individuals are available in a report edited by Hirai (2004).

We used the following taxonomic criteria for the taxa used in this study:

1. *H. agilis agilis* (Cuvier 1821), the mountain agile gibbon of Sumatra: the pelage of this taxon is buff, reddish-orange, reddish-brown, brown, or black with white cheek patches that connect at the chin and brow. The female's brow is white and not always divided in the middle.
2. *H. agilis unko* (Lesson 1840), the lowland agile gibbon of Sumatra: this taxon possesses few characteristics that reliably distinguish it from *H. a. agilis*. The cheek patches are creamy white to a grizzled white, sparse, and do not connect at the chin or brow. The adult female's brow marking is thin and short and well separated in the middle. Some specimens have a lumbar region that is paler than the rest of the body hair.
3. *H. albibarbis* Lyon 1911, the Bornean white-bearded gibbon: this taxon is light brown, with dark-brown to brown-black underparts, hands, legs, and cap, with a white brow, and a buff lumbar region. This taxon has black fingers and toes. Groves (2001) classified this group as a different species from Sumatran agile gibbons and Mueller's Bornean gibbons.

4. *H. muelleri* (Martin 1841), Mueller's Bornean gibbon: adult male and female are identical in coat color, which varies from gray to gray-brown or blackish. The hair of the adult male's genital tuft is 25 mm long (Marshall and Sugardjito 1986) and is typically darker than the body hair. Infant's coats are lighter than their parents'. This species lacks a uniform appearance in areas of geographic overlap between Mueller's Bornean gibbon subspecies. Marshall and Sugardjito (1986) describe three subspecies: Eastern Mueller's gibbon (*H. m. muelleri*), Abbott's gray gibbon (*H. muelleri abbotti*), and Northern Mueller's gibbon (*H. muelleri funereus*). Samples of this species collected in Kalimantan in this study were all from Eastern Mueller's gibbons. This subspecies is pale gray or gray-brown with a black cap, ventrum, hands, feet, and inner aspects of the limbs, and has a thick white brow.

Taxonomic identification and original locality (wild or captive born) of the 57 gibbons studied are listed in Table 3.1, together with morphs of chromosome 8 and haplotypes of DNA markers. The samples were collected in west Sumatra (Padang, Payakumbuh, Solok Selatan, Kunbang Tungkek, Bukit Tinggi, Panti, and Pasaman Timur), in central Kalimantan (Palangkaraya and Pangkalanbun) and in south Kalimantan (Banjarmasin, Banjar Baru, and Martapura).

Chromosomes

Gibbons of the genus *Hylobates* have 44 chromosomes, and are polymorphic for three pericentric inversions in chromosome 8 (a, b, c), which are shared by several species (Stanyon et al. 1987). Recently, a unique chromosome variation, a translocation between chromosomes 8 and 9 (van Tuinen et al. 1999), was found in the genus *Hylobates*. This variation was confirmed to be a whole-arm translocation between chromosomes 8 and 9 (WAT8/9) by chromosome painting analysis (Hirai et al. 2003). In combined data from previous studies by van Tuinen et al. (1999) and Hirai et al. (2003), the variant seemed to be predominant in Sumatran agile gibbons [*Hylobates agilis agilis* (79%) and *H. agilis unko* (63%)], but occurred in only 15% of Bornean agile gibbons (Hirai et al. 2003). That is, Sumatran agile gibbons (*H. a. agilis* and *H. a. unko*) had WAT8/9 about five times more frequently than Bornean agile gibbons (*H. albibarbis*). We initiated this project to further investigate this apparent pattern, since previous studies included insufficient samples of Sumatran taxa.

As shown in Table 3.1, in this study, only animals that were identified as Sumatran agile gibbons had the WAT8/9 translocation as a polymorphism, while it was not observed in Bornean agile gibbons (*H. albibarbis*) or Eastern Mueller's gibbons (*H. m. muelleri*) (Hirai et al. 2005). A three-color FISH technique using human chromosome paints disclosed that WAT8/9 was present in all 17 Sumatran agile gibbons studied (4 heterozygotes and 13 homozygotes) (Table 3.1), and that it was a translocation between morphs 8c and 9 (Fig. 3.2). WAT8/9 appears to be restricted to the Sumatran taxon.

Table 3.1 Sample and genetic data of *Hylobates agilis* and *H. muelleri* collected from Sumatra and Kalimantan

ID	Sex	Ori	Fac	S/SS	MC8	mt	TSPY	ID	Sex	Ori	Fac	S/SS	MC8	mt	TSPY
1	M	S. w	pet	AGU	8ab/8c'	AG1	AG	26	F	K. w	pet	AL	8c/8c	AL	-
2	M	S. w	pet	AGU	8c'/8c'	AG2	AG	27	F	K. w	pet	AL	8c/8c	AL	-
3	M	S. w	zoo	AGU//A	8c'/8c'	AG1	AG	28	F	K. w	pet	AL	8ab/8c	AL	-
4	M	S. w	pet	AGU	8c'/8c'	AG1	AG	29	M	-	zoo	AL	8ab/8ab	AL	AL
5	F	S. w	pet	AGU	8c'/8c'	AG2	-	30	M	c	pet	AL	8ab/8c	AL	AG
6	F	S. w	pet	AGU	8ab/8c'	AG2	-	31	M	c	pet	AL	8ab/8c	AL	AL
7	F	S. w	pet	AGU//A	8c'/8c'	AG2	-	32	M	c	pet	AL	8c/8c	AL	AL
8	F	S. w	pet	AGU//A	8c'/8c'	AG2	-	33	F	c	pet	AL	8c/8c	AL	-
9	F	S. w	pet	AG	8c'/8c'	AG1	-	34	F	c	pet	AL	8ab/8ab	AL	-
10	F	S. w	pet	AGU	8c'/8c'	AG1	-	35	F	c	pet	AL	8c/8c	AL	-
11	M	c	zoo	AGU	8c'/8c'	AG1	AG	36	F	c	pet	AL	8ab/8c	AL	-
12	M	c	zoo	AGU	8c'/8c'	AG1	AG	37	F	c	zoo	AL	8ab/8c	AL	-
13	M	c	zoo	AGU	8c'/8c'	AG1	AG	38	M	K. w	pet	MU	8ab/8c	MU	MU
14	M	c	zoo	AGU//A	8ab/8c'	AG2	AG	39	M	K. w	pet	MU	8c/8c	MU	MU
15	M	c	zoo	AGU	8c'/8c'	AG1	AG	40	M	K. w	pet	MU	8ab/8c	MU	MU
16	F	c	zoo	AGU	8ab/8c'	AG1	-	41	M	K. w	pet	MU	8ab/8ab	MU	MU
17	F	c	zoo	AGA	8c'/8c'	AG1	-	42	F	K. w	pet	MU	8c/8c	MU	-
18	M	K. w	pet	AL	8c/8c	AL	AL	43	F	K. w	pet	MU	8ab/8ab	MU	-
19	M	K. w	pet	AL	8ab/8ab	MU	MU	44	F	K. w	pet	MU	8ab/8c	MU	-
20	M	K. w	pet	AL	8c/8c	AL	AL	45	F	K. w	pet	MU	8ab/8ab	MU	-
21	M	K. w	pet	AL	8ab/8ab	AL	AL	46	F	K. w	pet	MU	8ab/8ab	MU	-
22	M	K. w	pet	AL	8ab/8c	AL	AL	47	F	K. w	pet	MU	8ab/8ab	MU	-
23	M	K. w	pet	AL	8ab/8c	AL	AL	48	M?	K. w	pet	MU	8ab/8c	MU	-
24	F	c	pet	AL	N	AL	-	49	M	c	zoo	MU	N	AL	N
25	F?	K. w	pet	AL	8ab/8c	AL	-	50	M	c	pet	MU	8ab/8c	MU	MU

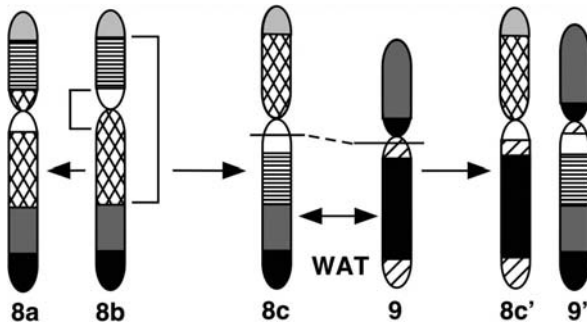


Fig. 3.2 Schematic illustration of the whole-arm translocation (WAT) between chromosomes 8 and 9 found in Sumatran agile gibbons. Each *patch* or *shade* element shows a block stained with the same human chromosome painting probe. The *brackets* indicate the breakpoints of the inversions. The *fine bar* indicates the breakpoints of the translocation. The *numbers* indicate the chromosome, and the *letters* refer to the morph of the chromosome. The *arrows* indicate the directions of chromosome changes. The *double arrowheads* show the exchange of chromosome arms

8a and 8b are other morphs that acquired different inversions, respectively, from 8c (see Fig. 3.2). These two morphs could not always be discriminated in all samples in the painting analysis of this time because of their similarity, though they were easily distinguishable from 8c, so for the purposes of this study we combined them and refer to either 8a or 8b as 8ab. Heterozygotes for WAT8/9 may include these other morphs of chromosome 8, that is, 8ab8c'99' and 8c8c'99'. Both of the heterozygous pairs could form quadrivalents in meiosis-I as shown by van Tuinen et al. (1999). Chromosome composition data deduced from painting (Fig. 3.2) suggest that the former heterozygote is more complicated than the latter. In general, the more complicated chromosome pairing would induce more meiotic non-disjunction, resulting in lower fecundity. However, gibbons investigated in three previous studies showed only the former pairing (8ab8c'99') (van Tuinen et al. 1999; Hirai et al. 2003; Hirai et al. 2005). Researchers have identified 13 individuals with 8ab8c'99' (not including offspring of captive parents with the same chromosome pairing), but none with 8c8c'99'. This suggests that morphs 8c and 8c', which ought to be most similar, may contain elements that make them incompatible with each other. However, the data are not yet sufficient to confirm this, and if such incompatibility exists, the mechanism is unknown.

DNA Analyses

To clarify molecular phylogenetic relationships between Sumatran and Bornean agile gibbons and Eastern Mueller's gibbons, we sequenced the ND4-ND5 region of mitochondrial (mt) DNA and the testis-specific protein Y-encoded

(TSPY) gene from blood. ND4-ND5 sequences (1039 bp) were amplified using two primers (L12686 and H12752R) described in a previous study (Hayashi et al. 1995), and sequenced with BigDye (R) Terminator Ver. 3.0 cycle sequencing kit (Applied Biosystems) (Tanaka et al. 2004). We amplified and sequenced the TSPY gene (739 bp) in nine Sumatran agile gibbons, eight Bornean agile gibbons, and six Eastern Mueller's gibbons using the primers described previously (Kim et al. 1996). We aligned the sequences with CLUSTAL X 1.81 (Thompson et al. 1997) and conducted phylogenetic analyses with PAUP* (Swofford 2003). We drew a network of TSPY haplotypes with TCS (Clement et al. 2000). We also conducted population genetic analyses of the relationship between the three taxa using 14 microsatellite loci (D02S1777, D05S0807, D09S0302, D10S1432, D14S0255, D17S0804, D20S0206, D07S1826, D01S0533, D03S1768, D07S0821, D13S0765, D13S0788, D14S0306) (Hayano et al. unpublished). Analysis of molecular variance (AMOVA, Excoffier et al. 1992) was conducted with ARLEQUIN (Schneider et al. 2000). We calculated F_{ST} distances to measure the extent of genetic distance between groups using the variance of genotypic frequencies in 195 independent alleles. As these data will be described in detail elsewhere, we will only briefly summarize them here.

Clustering analyses of the 40 mtDNA haplotypes found in 52 individuals suggest that Sumatran agile gibbons consist of two distinct groups (*agilis* 1 and *agilis* 2), and that *agilis* 1, *agilis* 2, *albibarbis*, and *muelleri* form a separated lump cluster. *H. albibarbis* and *H. m. muelleri* fall phylogenetically between the *agilis* 1 and *agilis* 2 clusters. However, the two *agilis* clusters do not seem to be in accordance with the subspecies of Sumatran agile gibbons (*agilis* and *unko*) (Tanaka et al. 2004 and unpubl.). On the other hand, 8 TSPY haplotypes were found and network analyses show clear separation of Sumatran agile gibbons (including *agilis* 1 and *agilis* 2) from Bornean agile gibbons (*H. albibarbis*) and Eastern Mueller's gibbons (*H. m. muelleri*) with 2–7 and 6–10 base pair differences, respectively (Fig. 3.3) (see also Tanaka et al. 2004; Hirai et al. 2005).

Six individuals (19, 30, 48, 52, 55, 56) showed mismatches between identifications by pelage pattern and by mtDNA or TSPY or both (Table 3.1). Mismatches detected included *albibarbis* (pelage) – *muelleri* (mtDNA) – *muelleri* (TSPY); *albibarbis* – *albibarbis* – *agilis*; *muelleri* – *albibarbis* – undetected; *muelleri* – *agilis* – *agilis*; *albibarbis* – *muelleri* – none; and *albibarbis* – *muelleri* – undetected. These discrepancies may result from interspecific or intersubspecific hybridization that is likely to cause misidentification, because mtDNA (inherited maternally) and TSPY (inherited paternally) originating from different species are observed in some of these individuals. These cases emphasize the point that morphological and genetic analyses of the same animal are usually required in phylogenetic or conservation studies of gibbons.

The mtDNA and TSPY divergences were confirmed by a genetic distance analysis with microsatellite DNA genotypes. Using 12 microsatellite loci with a total of 195 alleles, we calculated the F_{ST} value and tested for significance using 5000 permutations by AMOVA (Excoffier et al. 1992; Schneider et al. 2000). The AMOVA result indicated significant genetic differences among the three

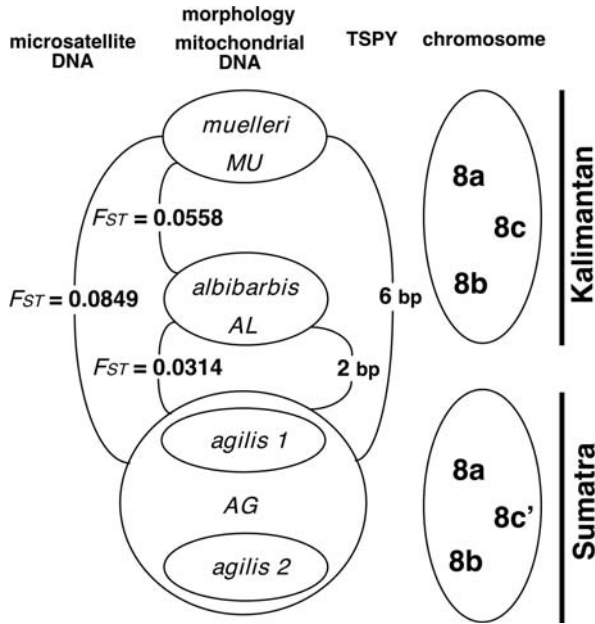


Fig. 3.3 Relationships among four genetic parameters-chromosome, mitochondrial DNA, TSPY, and microsatellite DNA in populations of three species, *H. muelleri muelleri* (*muelleri*), *H. agilis albibarbis* (*albibarbis*), and *H. agilis* (*agilis*) identified with pelage patterns. Network of TSPY differentiation was drawn fine black lines and circles. An interval bar between circles indicates one base pair substitution. Solid circles show haplotypes of TSPY found in the present study, and blank ones indicate intermediate haplotypes undiscovered so far. For the details see text

groups ($F_{ST} = 0.053$, $p < 0.001$). Further, significant pairwise F_{ST} values ($p < 0.001$) were found in all three pairwise comparisons: between Sumatran and Bornean agile gibbons (0.0314), Bornean agile gibbons and Eastern Mueller’s gibbons (0.0558), and Sumatran agile gibbons and Eastern Mueller’s gibbons (0.0849). These results suggest that the three populations are genetically distinct from each other (Fig. 3.3, see also Hirai et al. 2005).

Discussion

We have examined the genetic and morphological features of more than 100 animals of the genus *Hylobates* so far. One point highlighted by our investigations is that knowing the original collection locality for specimens used in genetic monitoring research in gibbons is extremely important, since morphological identification is difficult (van Tuinen et al. 1999). While we were careful in verifying the collection localities for our samples, we found discrepancies

between morphological and molecular identifications in six individuals. Most of these are probably caused by interspecific or intersubspecific hybridization, which is comparatively difficult to identify morphologically. However, in our experience, careful photography can sometimes aid in the identification of gibbons in zoological institutions. Therefore, interdisciplinary investigations of morphology and genetics are required to monitor and reduce misidentification of captive animals. Results from such studies should help to overcome deficiencies in the classification of gibbons. The mtDNA ND4-ND5 as well as D-loop regions are probably the best molecular methods for identifying captive gibbons at present, though development of appropriate nuclear DNA markers to detect introgression will be required for more intensive genetic studies. The inclusion of vocalization studies may be helpful for species and subspecies identification and for exploration of phylogenetic relationships among gibbon taxa. Using this more holistic approach may allow researchers to obtain more solid data on the evolution of small apes, although data based on DNA and chromosomes have not always yielded consistent results (Fig. 3.3).

Hitherto, genetic studies of gibbons have generally relied on samples from captive-born animals or animals of unknown origin, because of the ease of obtaining blood samples from zoological institutions rather than from pets of known origin or from wild animals. Captive gibbon management is difficult, due to identification problems resulting from the complexities of their morphology, and to inadvertent or unwitting hybridization (van Tuinen et al. 1999). Investigations using zoo samples can thus produce conflicting conclusions, because of the possibility that individuals appearing to belong to the same species actually have distinct genetic structures from different species due to hybridization or misidentification. In our previous investigation, we showed that some individuals of the genus *Hylobates* that have been reared in zoological institutions were misidentified. For example, one of us (ARM) correctly re-assigned captive individuals, from *H. klossii* to *H. agilis unko*; from *H. moloch* to *H. muelleri* and *H. agilis unko*; and from *H. muelleri* to *H. albibarbis*, etc. (Hirai et al. 2003). Another study reported a similar experience (van Tuinen et al. 1999). Such re-identifications, and the accumulation of data from numerous individuals, allowed us to estimate the geographical distribution of the WAT8/9 translocation (Hirai et al. 2003), and a project using gibbons of known origin revealed a new variant marker chromosome that identified Sumatran agile gibbons as an ESU (Hirai et al. 2005). To date, our samples have been limited to central and western Sumatra, and have confirmed that the presence of WAT8/9 distinguishes Sumatran agile gibbons in this area from other gibbon taxa. Complete sampling from across their range on Sumatra will be necessary to determine the geographic extent of occurrence of this genetic variant.

Do chromosomal variations drive speciation events? A theoretical analysis of chromosome change and species differentiation suggested that speciation without karyotype alteration predominates in mammals (Imai 1983). Imai (1983) concluded that parapatric distributions of karyotypically distinct populations are a transitional step in karyotype substitution. On the other hand, the

stasipatric speciation model states that chromosome changes can be strongly implicated in driving a speciation event by creating a barrier to gene flow, rather than being only a remnant of adaptation by directional selection (White 1978). The WAT8/9 translocation polymorphism, found only in Sumatran agile gibbons, is interpreted as a transitional step toward its fixation by genetic drift. Microsatellite DNA analysis suggested, based on a significant heterozygote deficit, that the sample population of Sumatran agile gibbons has experienced a population bottleneck (A. Hayano, unpubl. data). During the bottleneck, the chromosome alteration may have occurred in a small population, and afterwards may have spread rapidly by genetic drift during an abrupt increase in population size. WAT8/9 apparently occurred on Sumatra after the geographical isolation of Sumatra and Borneo, and thus could be rapidly fixed on Sumatra. However, the chromosome change does not distinguish the subspecies *H. agilis agilis* and *H. agilis unko*, as both subspecies have the same alteration (van Tuinen et al. 1999; Hirai et al. 2005). Data from chromosome and DNA analyses reveal that the two Sumatran subspecies of agile gibbons appear to belong to a single species, *H. agilis*, though they display distinct pelage patterns. Thus, genetic analyses are pivotal tools to define population structure, especially of gibbons with similar pelage patterns.

Will or did the WAT8/9 translocation drive the evolution of Sumatran agile gibbons? The 8c' element of the translocation appears to have some incompatibility with 8c, which is a direct ancestor of the alteration. The incompatibility might have resulted in selection against chromosome 8c in Sumatran populations of agile gibbons, because other populations of *H. albibarbis* and *H. m. muelleri* without 8c' still include 8c (Fig. 3.3). If this is indeed the case, then 8c will be eliminated on Sumatra in the future or may already have been eradicated, because it was not observed in the present study. Chromosomal changes such as the translocations described here could result in lowered fitness when in the heterozygous condition because of problems in meiosis. However, Cronin et al. (1984) point out that chromosome variants in gibbons that are generally socially monogamous could become homozygous more rapidly than in animals with a different social structure and mating system. If variants such as 8c' become homozygous in a population, they recover the same fitness as the ancestral wild-type homozygote. Accordingly, it seems that the Sumatran population of agile gibbons is evolving rapidly toward an 8c' population as a result of the unique social structure of gibbons. The mechanism of fixation of WAT8/9 is probably a good example of chromosome evolution in gibbons by numerous translocations. Chromosome evolution by translocation may occur more readily in primates with a monogamous mating system than with the polygamous and promiscuous mating systems found in other primate groups. That is, the mating system of gibbons may be tightly linked with chromosome evolution, though there is increasing evidence that the social structure in gibbons is not necessarily as rigid as has been presumed (Hirai et al. 2005).

We have postulated that migration of *muelleri* and *agilis* (or *albibarbis*) gibbons from Sumatra to Borneo may have occurred twice, based on our

genetic data and geographic changes in the glacial period (Fig. 3.3). However, Groves (1972) suggested an alternative route from Indochina to Borneo and then from Borneo to Sumatra during the Pleistocene to explain the distribution pattern of gibbons in relation to the geographic data. As our hypothesis was proposed based on data on chromosome change and genetic distance between the three taxa (*H. agilis*, *H. albibarbis*, and *H. m. muelleri*) of Sumatra and Borneo, cladistic calibration with molecular data for ancestral taxa is required to determine the direction of migration of gibbons in Sundaland.

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