Taxonomy, geographic variation and population genetics of Bornean and Sumatran orangutans

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Abstract and Keywords
This chapter reviews the published data and discusses the taxonomy and population genetics of orangutans. The orangutan was traditionally classified as two separate subspecies, *Pongo pygmaeus pygmaeus* in Borneo and *P. p. abelii* in Sumatra. Recent molecular data have suggested a re-classification into two separate species: *P. pygmaeus* in Borneo and *P. abelii* in Sumatra. Moreover, three subspecies have been described on Borneo Island: *P. p. pygmaeus* in Sarawak and west Kalimantan, *P. p. morio* in Sabah and east Kalimantan and *P. p. wurmbii* in central and south Kalimantan. Despite this, little is known about the intra-subspecific variation between isolated Bornean populations and among the Sumatran...
populations. More data are needed, which should include a large sampling of all geographically separated populations in Borneo and Sumatra in order to provide a more complete genetic information database.

Keywords: geographic isolation, molecular, non-invasive genetics, population genetics, taxonomy

1.1 Introduction
The orangutan was first described in the early seventeenth century by two Dutch physicians, Jacob de Bondt and Nicholaas Tulp, and then assigned a taxonomic name, *Simia satyrus*, by Carl von Linné, a name which was subsequently changed to *Pongo pygmaeus* in 1927 by the International Commission on Zoological Nomenclature (for more details, see Rijksen and Meijaard 1999). Despite a wide Pleistocene distribution in South East Asia and mainland Asia, including areas between Vietnam, northern India and southern China (Hooijer 1948; Kahlke 1972; von Koenigswald 1982; Tougard and Ducrocq 1999; Bacon and Long 2001), wild orangutan populations are today found only in Northern Sumatra and Borneo. These two islands are isolated from each other by the South China Sea, an isolation that has been effective for at least 8000 years (Harrison et al. 2006). Sub-fossil orangutans are limited but
finds dated at 30,000–40,000 before the present (BP) have been discovered in both Sumatra and Borneo (Smith and Pilbeam 1980).

Different approaches and molecular (mainly DNA-based) markers have been used to estimate the divergence time between Bornean and Sumatran orangutans, leading, unsurprisingly, to somewhat differing estimates (see Table 1.1). It appears from (p.2)

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<tr>
<th>Divergence time estimate (Ma)</th>
<th>Molecular marker used</th>
<th>Reference</th>
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<tr>
<td>1.1</td>
<td>MtDNA control region (278bp)</td>
<td>Warren et al. 2001</td>
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<tr>
<td>0.6–3.4</td>
<td>MtDNA control region</td>
<td>Gagneux et al. 1999b</td>
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<td>Non-coding Xq13.3 DNA sequences (10,000bp)</td>
<td>Kaessmann et al. 2001</td>
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<td>MtDNA RFLP, nuclear minisatellite loci and mt 16S ribosomal RNA sequences</td>
<td>Zhi et al. 1996</td>
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<td>MtDNA ND5 gene</td>
<td>Zhang et al. 2001</td>
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<tr>
<td>2.7–5.0</td>
<td>MtDNA genes and 2 nuclear genes</td>
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<td>3.5</td>
<td>MtDNA COII sequences</td>
<td>Ruvolo 1996</td>
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<tr>
<td>5.1±1.3</td>
<td>Complete mt DNA genome</td>
<td>Arnason et al. 1996</td>
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<tr>
<td>3.5–4.7</td>
<td>Complete mt DNA genome</td>
<td>Raaum et al. 2005</td>
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these studies, then, that the divergence between the Bornean and Sumatran orangutans could have taken place any time between 0.6 and 6.4 million years (Ma) ago.
Until recently, orangutans were classified into two subspecies (or races, see van Bemmel 1968; Jones 1969), the Bornean (*P. pygmaeus pygmaeus*) and the Sumatran (*P. p. abelii*), rather than two separate species, despite differences in hair length, structure and color (red to deep maroon or blackish brown for the Bornean orangutan, lighter-colored, rusty red or light cinnamon for the Sumatran orangutan), distribution of facial hair, size and shape of the cheek flanges and the throat sac in males, body build, presence or absence of a nail on the big toe, and various craniodental characteristics (van Bemmel 1968; Jones 1969; MacKinnon 1973; Mallinson 1978; Groves 1986; Courtenay *et al*. 1988; Uchida 1998a; Delgado and van Schaik 2000). These morphological distinctions are further supported by karyotypical data, with the two orangutans differing cytogenetically by a pericentric inversion in chromosome 2 (Seuanez *et al*. 1979), and by molecular data (Janczewski *et al*. 1990; Ryder and Chemnick 1993; Ruvolo *et al*. 1994; Xu and Arnason 1996; Zhi *et al*. 1996). More specifically, the analysis of mitochondrial DNA has led some authors to suggest that the divergence levels between the two subspecies was enough to elevate them to species (Xu and Arnason 1996). However, this was challenged by Muir *et al*. (1998, 2000), who suggested a more complex orangutan evolutionary scenario (e.g., three distinct lineages in Sumatra). Recently, Fischer *et al*. (2006) sequenced multiple intergenic autosomal regions totalling 16,000 BP in 10 Bornean and 6 Sumatran orangutans and 22,400 BP in western (*Pan troglodytes verus*), central (*P. t. troglodytes*) and eastern (*P. t. schweinfurthii*) chimpanzees and in bonobos (*Pan paniscus*). They analyzed these regions with reference to homologous data from humans and gorillas and found that orangutans have the highest diversity (as found in other studies) but that the extent of genetic differentiation among the two orangutan subspecies was only comparable to that seen among human populations. They therefore questioned the validity of the ‘subspecies’ concept for orangutans (as well as for chimpanzees). The high diversity in orangutans is probably due to the multiple origins of orangutan populations that repopulated Borneo and Sumatra after successive glacial maxima (Muir *et al*. 2000). Nevertheless, differences between orangutan subspecies in Sumatra and Borneo are shown by mitochondrial restriction mapping, mtDNA sequences and DNA hybridization to be even greater than those among gorillas (Ryder and Chemnick 1993; Ruvolo *et al*. 1994; Xu and Arnason 1996). It is also apparent...
that Sumatran orangutans have higher nucleotide diversity than Bornean orangutans, and that orangutans are more
diverse than African (p.3) apes and humans (see also Fischer
et al. 2006). Higher polymorphism for the Sumatran taxon is
found in almost all other orangutan genetic data sets
(Kaessmann et al. 2001; Noda et al. 2001; Muir et al. 2000;
Warren et al. 2001; Zhang et al. 2001; Zhi et al. 1996) and may
reflect past geological events and a higher historical effective
population size in Sumatra (Muir et al. 2000). Steiper et al.'s
(2005) ?-2 globin data also support this interpretation. Using
two different modeling approaches, Steiper (2006) re-analyzed
most of the available molecular data and concluded in this
meta-analysis that there was support for a divergence between
Bornean and Sumatran orangutans 2.7–5 million years ago.

This deep split suggests that Pleistocene events, such as the
cyclical exposure of the Sunda shelf and the Toba volcanic
eruption (Rampino and Self 1992) did not have a major impact
on the divergence of Bornean and Sumatran orangutans. Note
that this does not mean that they did not have other effects on
the patterns of diversity within both species. For instance,
pairwise nucleotide mismatch distributions, typically used to
detect and date past population expansions using mtDNA,
suggest that Bornean orangutans underwent a population
expansion, beginning 39,000–64,000 years ago, i.e. after the
Toba eruption, which was c. 70,000 years ago. However, no
such signal was detected in Sumatran populations.

Currently, if one refers to the most recent Asian primate
classification published by Brandon-Jones et al. (2004), one
species is recognized for the island of Sumatra, P. abelii, and
two subspecies for the island of Borneo, the Western Bornean
orangutan (P. p. pygmaeus) in west Kalimantan, north of the
Kapuas River in Indonesia and in west Sarawak in Malaysia;
and the Southern Bornean orangutan (P. p. wurmbii) in south-
west Kalimantan, between the Kapuas and Barito Rivers, in
Indonesia. They also recognize a separate population of P.
pygmaeus in East Kalimantan (south to the Mahakam River in
Indonesia) and in Sabah in Malaysia. In the recent Orangutan
Population and Habitat Viability Assessment (Singleton et al.
2004), this ‘population’ was designated as a third Bornean
subspecies, P. p. morio.
However, if we were to adopt a strict biological species concept—groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups—(Mayr 1963) and, knowing that Bornean and Sumatran orangutans can interbreed in captivity and produce fertile offspring (de Boer and Seuanez 1982), the question arises: do we really have two different species? (Tangentially, it could be worth questioning the value of such a debate while orangutans on both islands may not survive the next 50 years if nothing is done to stop their ongoing decline). Alternatively, it is perhaps worth considering these simply as separate populations or management units, particularly since recent studies (Warren et al. 2001; Goossens et al. 2005a; Jalil et al. 2008) show that the genetic structure of orangutan populations is very complex and most probably shaped by geographical barriers such as mountain ranges and large rivers. Using the control region of the mitochondrial DNA for six different Bornean populations, Warren et al. (2001) identified four distinct subpopulations with particular regional diversity and geographic clustering: (1) Southwest and Central Kalimantan, (2) Northwest Kalimantan and Sarawak, (3) Sabah, and (4) East Kalimantan. If we compare these four subpopulations with the three subspecies described above, we have *P. p. pygmaeus* corresponding to (1), *P. p. wurmbii* corresponding to (2) and *P. p. morio* corresponding to (3) and (4). This structure strongly suggests that rivers have influenced the migration and the colonization of Borneo by orangutans and that they contributed to the shaping of genetic structure in orangutans over evolutionary timescales. This result is confirmed by recent studies carried out by ourselves, which we describe in more detail below (Goossens et al. 2005a; Jalil et al. 2008). The impact of large rivers on the genetic structure of great apes has also been shown in bonobos (*Pan paniscus*) by Eriksson et al. (2004).

Perhaps one of the most important points to make at this juncture is that unfortunately, only a few population genetic studies of orangutans have used samples collected in the wild, with precise origins known (but see Warren et al. 2001; Goossens et al. 2005a, 2006a) and the only two studies that included known-origin individuals were carried out in Borneo. It is therefore difficult to make any geographic comparison between populations, and (p.4) between the two islands. In this chapter, we summarize our work carried out in Borneo.
(Kinabatangan, Sabah) on population genetic structure, as well as work carried out by others in Sumatra and (predominantly) Borneo. Unfortunately, much more molecular data have been generated for at least two of the three other great apes, particularly for gorillas and chimpanzees but also, arguably, for bonobos, and we include a brief overview of these studies, where their results are potentially relevant to orangutans.
1.2 Sampling issues in orangutan genetic studies
The analysis of orangutan evolution, population structure, genetic diversity and migration/gene flow within the species range requires samples from individuals with known geographic origin and, where appropriate, across their present geographic range. Samples with reliable origin can only usually be collected in the wild, but non-habituated orangutans appear to be extremely difficult to sample, being semi-solitary, very elusive and shy, and almost exclusively arboreal. Therefore, most of the genetic studies carried out in the last 10 years have used blood, tissue and plucked hair samples from captive orangutans (most of them of wild origin) in zoos and rehabilitation centers (mainly from Kalimantan; Zhi et al. 1996; Muir et al. 2000; Kanthaswamy et al. 2001, 2006; Kanthaswamy and Smith 2002). Warren et al. (2000, 2001) used a mix of samples (blood and plucked hair) collected from rehabilitant animals and samples (shed hair) collected from night nests in the wild. To date, Goossens et al. (2005a, 2006a) have carried out the only population genetic studies exclusively using samples (shed hair and feces) collected from the wild (Lower Kinabatangan floodplain, Sabah, Malaysia).

The development of the polymerase chain reaction (PCR) (Saiki et al. 1985) has increased the potential of using minute amounts of DNA obtained from non-invasive samples such as shed hair collected in night nests and feces (containing sloughed epithelial cells from the colon), and has led to a number of studies on wild populations of great apes, mainly chimpanzees (Sugiyama et al. 1993; Morin et al. 1994; Gagneux et al. 1997, 1999a; Vigilant et al. 2001), bonobos (Gerloff et al. 1999; Eriksson et al. 2004), and more recently gorillas (Jensen-Seaman and Kidd 2001; Clifford et al. 2004; Lukas et al. 2004; Bradley et al. 2005). The use of non-invasive samples has its limitations, particularly when using nuclear DNA, because DNA extracted from hair or feces is often degraded and its analysis requires extreme precautions (see Taberlet et al. 1999; Goossens et al. 2000; Morin et al. 2001; Bradley and Vigilant 2002; Goossens et al. 2003; Nsubuga et al. 2004). Also, one should keep in mind that the total number of independent genetic markers that will be used is to a large extent limited and often the material cannot be used for future studies.
Altogether, the sampling issue is crucial but the difficulty to sample wild animals and the uncertainty resting on the origin of many captive individuals suggest that a major international effort should be carried out to increase the sampling effort, and find methods that allow us to genotype very large number of loci from non-invasive samples.

1.3 Genetic markers: advantages and limitations
The scientific question of interest will determine the choice of the molecular marker. The two most common markers used in population genetics are mitochondrial DNA and nuclear microsatellites.

1.3.1 Mitochondrial DNA
Mitochondrial DNA (mtDNA) is mainly characterized by: (1) its strictly maternal and haploid inheritance; (2) its high sequence polymorphism (occurring mainly in control region (or d-loop)); (3) the high number of copies per cell; and (4) the almost complete absence of recombination. This means that gene trees can be reconstructed from mtDNA data (this is not true for nuclear genes, over a long enough time period) and that all loci behave as a single genetic unit. MtDNA can therefore be used to infer maternal relationships in a population and female dispersal rates between populations. MtDNA sequences are also used to reconstruct phylogenetic relationships at different taxonomic (p.5) levels. This is made possible by the fact that the molecule has both slow- and fast-evolving genes. For instance, the control region, which is the only major mtDNA non-coding region, is typically used for phylogeographic or population-based studies because it evolves 10–20-fold faster than most available nuclear genomes. It should be added that selection acting on one mtDNA gene will influence the whole molecule as it does not recombine, contrary to statements sometimes found in the literature suggesting that the non-coding control region is unaffected by selection.

In the study of great apes and primates in general, mtDNA has been primarily used in phylogeny (Shoshani et al. 1996; Xu and Arnason 1996; Gonder et al. 1997; Gagneux et al. 1999b; Shimada et al. 2002; Jensen-Seaman et al. 2004; Newman et al. 2004; Guillén et al. 2005), phylogeography (Warren et al. 2001 in orangutans; Hofreiter et al. 2003; Clifford et al. 2004 in gorillas), and biogeography (Goldberg and Ruvolo 1997a, b; Goldberg 1998; Jensen-Seaman and Kidd 2001) and for identifying genetically distinct units for conservation (Gagneux
et al. 2001; Warren et al. 2001). It can also be used to examine genetic distinctiveness within populations, although as a maternally inherited marker, it is strongly affected by patterns of female philopatry and dispersal (see Hapke et al. [2001] for an example in the Eritrean hamadryas baboons [Papio hamadryas hamadryas]).

Although analysis of mtDNA sequence variation has been used to study the evolutionary relationships of populations, both within and across species, Thalmann et al. (2004) caution their use and describe ‘the presence of translocated pieces of mtDNA (”Numts“) in the nuclear genome of many taxa that may be mistaken for authentic organellar mtDNA’. More recently, Anthony et al. (2007) have investigated the problem of ‘Numts’ and in vitro (PCR-generated) recombination in lowland gorilla phylogeography. To our knowledge, such assessment of ‘Numts’ in orangutans has not been carried out yet.

1.3.2 Nuclear DNA (mainly microsatellites)

Microsatellites were first used as molecular markers in the late 1980s (Tautz 1989). They are simple sequence repeats found in the nuclear DNA and are present in thousands of copies scattered throughout the genome. They comprise repeated segments of two to six DNA bases and their variation is mainly derived through changes in the number of DNA repeats at any given location. They are most abundant in non-coding regions of the genome and they possess a high mutation rate (Hancock 1999; Zane et al. 2002). Microsatellites have been used in a number of primate and great ape species to determine the patterns of genetic diversity and genetic structure (e.g. Fredsted et al. 2005 in gray mouse lemur [Microcebus murinus]; Reinartz et al. 2000 in bonobos), as well as patterns of genetic paternity and/or relatedness in Hanuman langurs (Semnopithecus entellus) (Launhardt et al. 2001), in chimpanzees (Constable et al. 2001; Gagneux et al. 1999a; Vigilant et al. 2001), in bonobos (Gerloff et al. 1999), in mountain gorillas (Gorilla gorilla beringei) (Bradley et al. 2005), in Sumatran and Bornean orangutans (Utami et al. 2002; Goossens et al. 2006b) and patterns of gene flow (e.g. Gagneux et al. 2001 in chimpanzees).

1.3.3 Sex chromosomes
Sex chromosomes can be particularly interesting for the study of primates in their natural environment as they can provide DNA markers useful for the gender identification of animals that cannot be easily approached or identified (e.g. those with little sexual dimorphism and in infants). Human Y-chromosomal microsatellite markers have been identified and analyzed in non-human primates (Erler et al. 2004), providing useful polymorphic, sex-specific markers available for investigating questions in behavioral ecology such as male-specific patterns of dispersal, male reproductive strategies and mating system; as well as investigating evolutionary questions (see Lawson Handley et al. [2006] in Saudi-Arabian hamadryas baboons [Papio hamadryas hamadryas]). The amelogenin (single-copy nuclear gene) locus has been used to identify sex, as the X and Y homologous copies are sufficiently different to generate different band patterns in males and females. The amelogenin gene has been shown to be effective in closely related great apes (Bradley et al. 2001; Matsubara et al. 2005). (p.6) Unfortunately, the original method seemed ineffective in orangutans (Bradley et al. 2001; Steiper and Ruvolo 2003; B. Goossens personal observation) and other primate species (Ensminger and Hoffman 2002).

Recently, other simple PCR-based approaches have been proposed that work across many species including prosimians, Old World and New World primates (Fredsted and Villesen 2004; Di Fiore 2005; Villesen and Fredsted 2006). Basically, most of them rely on the amelogenin system, the zinc-finger protein gene, or the SRY locus. Depending on the type of material used (fecal versus fresh material, agarose versus acrylamide gel) one system or another may be more easily adapted (Villesen and Fredsted 2006).

1.3.4 Development of single nucleotide polymorphisms

Because (1) they allow to access variability across the whole genome, (2) they are abundant and widespread in coding and non-coding regions of the genome and (3) they allow multiple, independent estimates of phylogeny, single nucleotide polymorphisms (SNPs) could become the genetic marker of choice to study the population genetics of wild endangered species (Morin et al. 2004). The fact that they allow amplification of extremely small fragments (between 50 and 150 base pairs) makes them extremely useful when using degraded DNA from non-invasive samples. In primates, studies are still very limited. Smith et al. (2004) designed the first 15
SNPs from chimpanzees, derived from the Y chromosome and autosomal regions of the genome, but no studies yet seem to have been published in other non-human primates.

1.3.5 Whole genome amplification

The recent establishment of whole genome amplification such as multiple displacement amplification (MDA) (Dean et al. 2002) would bring another dimension to non-invasive genetics and to species conservation efforts since it allows the production of large quantities of whole genomic DNA from minute sources (shed hair, feces, urine, wadges or discarded food items) (Rönn et al. 2006). One of the many potential applications could be to compare primate genomes and provide a better understanding of their adaptation to their environment (see Enard and Pääbo 2004; Ryder 2005 for reviews).

1.4 Principles of population genetic data analysis

In this section we briefly describe the principles behind some of the methods typically used in population genetics, and in the studies presented below, without going into the details of every approach but with reference to some of the software required. Some general references would probably be useful (Chikhi and Bruford 2005; Excoffier and Heckel 2006). Typically, the first step of any such study requires populations to be sampled and typed or for data to be collected from the literature or from databases. In the second step the aim is to describe the patterns of genetic variation. In particular, the aim is to determine whether the populations sampled are genetically variable or not, whether allele frequencies vary between populations, and whether these differences follow some spatial trends. The third step is usually the most controversial and complex as it involves inferences and conclusions to be drawn. More specifically, the questions that are asked are: is it possible to use the observed patterns (or lack thereof) to favor a particular past demographic or selective scenario? Is it possible to quantify the relative importance of different evolutionary factors (migration events, genetic drift, population size changes, selective pressures, mutation and recombination events, etc.) that led to the present-day patterns? Can such events be detected, quantified and dated?

1.4.1 Measuring diversity within and between populations
Genetic diversity is usually measured using the number of alleles across loci, the observed heterozygosity, $H_O$, and the expected heterozygosity, $H_E$. $H_E$ represents the heterozygosity one would expect to observe if the sampled individuals have been produced by random mating in the previous generation, whereas $H_O$ represents the heterozygosity observed in the data. Differences between $H_O$ and $H_E$ thus measure departures from random mating (assortative mating, inbreeding or population structure) even though other causes such as selection or the presence of null (non-amplifying) alleles are potentially important. When multiple locus data are used it is important to determine whether the loci can be treated as (roughly) independent or whether some loci should be discarded because they are statistically linked (i.e. there is redundant information).

Linkage disequilibrium is thus estimated for all pairs of loci for each sample and for the whole data set. All these measures are typically measured within populations and hence inform us little about spatial patterns of simply genetic differences. To do that, different statistics need to be used.

The amount of population differentiation between populations is usually measured by using the $F_{ST}$ statistic, which varies between 0, when populations have the same alleles at the same frequencies, and 1, when populations have fixed different alleles. Geographical patterns can then be analyzed by looking at the correlation between pairwise geographical and genetic distances. Since pairwise distances among populations are not independent, the correlation between the two distance matrices is typically assessed by using a randomization approach, typically a Mantel test. Most of the measures mentioned only use information from frequency data. In other words, the values calculated do not depend on whether some alleles are more similar to each other than to the rest of the alleles. With molecular data such as DNA sequences or microsatellite loci, it is possible to quantify the level of similarity between alleles, and use this information. Thus, $H_E$ and $F_{ST}$ have been extended to account for distances between alleles. For instance, the nucleotide diversity $\pi$ is a measure of heterozygosity at the nucleotide level, as it measures the average number of differences between two DNA sequences. Another classical way of representing genetic diversity with DNA sequences is to plot the so-called mismatch distribution. This is a histogram of the number of differences
between DNA sequences across all pairs of sequences in a sample. The mismatch distribution is typically used to detect ancient expansions or population bottlenecks as its shape has been shown to be influenced by such demographic events. Most of the analyses can be performed using freely available software such as Genepop (Raymond and Rousset 1995), Arlequin (Schneider et al. 2000), PopGene (Yeh et al. 1997), DnaSP (Rozas and Rozas 1999; Rozas et al. 2003) or Genetix (Belkhir et al. 1996/1997) among many others.
1.4.2 Detection and quantification of demographic events

As noted above, the description of patterns is interesting because it should allow us to better understand the recent (or not so recent) evolution of the species of interest. Mismatch distributions have been extensively used with mtDNA data to determine whether signals of expansions can be detected and dated. Typically, it has been shown that in stable populations, the mismatch distribution is expected to be ragged with multiple modes, whereas populations that have been expanding from a relatively small population size are expected to produce unimodal (bell-shaped) distributions. In the latter case, simulations have shown that the mode can provide an approximate estimation of the start of the expansion. A number of other statistics such as Tajima's (1989) \( D \), Fu's (1997) \( F_S \) or Fu and Li's (1993) \( D^* \) and \( F^* \) have also been shown to be sensitive to demographic events, and are commonly used to detect bottlenecks and expansion events.

While these statistics are useful to detect demographic events, they cannot be easily used to quantify or date such events. Indeed, the same value of Tajima's \( D \) could be the result of many, say, expansion events, of varying magnitude and age. Recent years have thus seen the development of computationally intensive methods aimed at using the genetic data in a more efficient manner. Many of these methods use information from the full allelic frequency distribution, and are Bayesian. They aim at determining probability distributions for parameters of interest that have been implemented in the models. For instance, Beaumont (1999) developed a full-likelihood Bayesian method for a demographic model that assumes that a stable population of size \( N_1 \) started to decrease (or increase) \( t \) generations ago to the current population size, \( N_0 \). Without getting into too many technical details, he showed, based (p.8) on these assumptions, that it is possible to estimate the ratio of present to ancient population size. The method uses highly computational approaches, which means that it is very slow. However, the advantage over previous methods is that it allows quantification of the population increase or decrease. This method was later expanded by Storz and Beaumont (2002), who showed how to quantify the effective population sizes \( N_0 \) and \( N_1 \), independently, as well as the time \( T \) since the population change (in generations). The two methods are implemented in the programs msvar 0.4 and
msvar 1.3, respectively, and have been used by Goossens et al. (2006a) for orangutan populations.

Other full-likelihood methods have been developed for different demographic models such as admixture (Chikhi et al. 2001, applied for humans by Chikhi et al. 2002), or population divergence (Wakeley and Hey 1997), applied in humans by Hey (2005), in bonobos and chimpanzees by Won and Hey (2005), and in orangutans by Steiper (2006). In the latter approach it is possible to date a divergence event while allowing for the daughter populations to have different effective sizes, both from each other and from the ancestral populations. It is worth pointing out that most of these full-likelihood methods are very slow and difficult to apply to large data sets. They often require some adjustment of parameters and simulations may take weeks and may need to be repeated. The outputs are also not always user-friendly and require some expertise to provide useful results.

It may be worth noting that most of the DNA-based studies typically use mtDNA. This is of course due to the fact that it is easy to characterize, even for degraded samples. However, it should be remembered that selection can also affect mismatch distributions and all the statistics mentioned above in ways that are similar to demographic events. Thus, it is on principle impossible to reject selection as a possible cause for signals of, say, expansions, unless they can be confirmed with other (nuclear) loci.

1.5 Orangutan genetic studies: where are we now?
This section will be divided in two subsections: 1.5.1, Population genetics and phylogeography of orangutans in Borneo (Warren et al. 2000, 2001; Kanthaswamy and Smith 2002; Goossens et al. 2005a, 2006a; Kanthaswamy et al. 2006; Jalil et al. 2008). So far, little work has been done in Sumatra (but see Kanthaswamy et al. [2006] on a limited number of samples). Section 1.5.2 considers comparison with other great apes (gorillas, chimpanzees, bonobos).

1.5.1 Phylogeography and population genetics of Bornean orangutans
So far, six comprehensive genetic studies have been published, four on a large scale (Warren et al. 2000, 2001; Kanthaswamy and Smith 2002; Kanthaswamy et al. 2006) and two on a
smaller scale (Goossens et al. 2005a, 2006a). In the following we shall also mention a study by Jalil et al. (2008). To our knowledge, no similar population genetic studies have been published for Sumatra.

1.5.1.1 Large scale: intrasubspecific variation of Bornean orangutans (Warren et al. 2001)

In their study, Warren et al. (2001) used blood and hair samples from 41 Bornean and 5 Sumatran orangutans sampled in six and one population, respectively. About 50% of the samples were collected from wild individuals and 50% from rehabilitants. About 240 base pairs of the mtDNA control region were sequenced and a maximum-likelihood tree was constructed and population pairwise $F_{ST}$ values and percentages of sequence divergence were estimated. The six regions sampled in Borneo clustered into four significantly differentiated populations: (1) Southwest and central Kalimantan, (2) Northwest Kalimantan and Sarawak, (3) Sabah, and (4) East Kalimantan. They are estimated to have diverged approximately 860,000 years ago. The authors therefore proposed that natural geographic barriers (such as large rivers) may have shaped the isolation and colonization of the four regions. They also emphasized that the Bornean and Sumatran orangutans were possibly reproductively isolated long before the two islands were geographically isolated by increasing sea levels in the Late Pleistocene. They also found high levels of genetic diversity among the different regions (and apparently higher levels than in other (p.9) apes) and hence argued that Bornean orangutans had not undergone a severe genetic bottleneck.
1.5.1.2 Microsatellite DNA variation in Bornean orangutans

(Warren et al. 2001)

In this paper, Warren et al. determined the extent of genetic variation within the Bornean populations of orangutans, using five polymorphic human-derived microsatellite loci, and 96 blood samples from individuals of known origin from East (n = 68), West (n = 21) and Central Kalimantan (n = 7). The results suggested that the East and West Bornean populations had not diverged significantly. However, the authors found clear deviations from the Hardy–Weinberg equilibrium and explained the excess of homozygotes as the consequence of differences in allele frequency between populations that are isolated and subjected to inbreeding (although this could also have been due to hidden local population structure). Moreover, Warren et al.‘s (2000) results indicate that the orangutans in East and West Kalimantan have similar genetic backgrounds, but form populations that are subjected to genetic drift.
**1.5.1.3 Population subdivision and gene flow among wild orangutans (Kanthaswamy and Smith 2002)**

In this paper, the authors used seven microsatellite loci to assess genetic variability among populations of orangutans from Borneo and Sumatra. Samples were from wild-caught or zoo animals (19 Sumatran and 73 Bornean individuals). The Sumatran individuals were from Ketambe and Suaq Balimbing, Gunung Leuser National Park. The Bornean orangutans were from Central Kalimantan (19), Sarawak (14), East Kalimantan (Kutai National Park, 13) and Sabah (27). Their results indicated substantial genetic differentiation among the Bornean subpopulations (consistent with Warren et al.'s (2001) results and with our results in the Kinabatangan area, see below, but in disagreement with the limited differentiation observed by Warren et al. 2000). Interestingly, Kanthaswamy and Smith explain the genetic similarity between Central Kalimantan and Sumatran orangutans as the result of the effects of the Indonesian government's inter-island translocation programs. It should be said that their study is based on a limited number of samples that were not from natural populations. It is thus not clear to what extent this conclusion would extend to natural populations, and it would be premature to assume that translocated individuals from Sumatra have had a strong genetic impact in the Central Kalimantan population. However, rehabilitants that were released in Ketambe are known to have surviving offspring and successfully breed (Utami et al. 2002). It should be noted though that the Central Kalimantan orangutans could be the closest of all Bornean orangutans to the Sumatran orangutans, since gene flow between the two islands most probably took place between Borneo and the southeast corner of Sumatra (Harrison et al. 2006).
1.5.1.4 Inferring Pongo conservation units: a perspective based on microsatellite and mitochondrial DNA analyses (Kanthaswamy et al. 2006)

In this study the authors used tissue and blood samples from 50 wild-born Bornean orangutans from rehabilitation centres and blood samples from 20 zoo orangutans of Sumatran origin. They analyzed partial sequences of four mitochondrial genes and nine autosomal microsatellite loci to investigate the population structure within Borneo and the genetic distinctiveness between Bornean and Sumatran orangutans. Their data showed that Bornean orangutans consist of two genetic clusters (hence confirming the results of Kanthaswamy and Smith [2002])—the western and the eastern clades—and that each species exhibits relatively distinct mtDNA and nuclear genetic distributions that they attribute to genetic drift, with the level of mtDNA genetic diversity in Sumatra being tenfold higher than that in Borneo (see also Muir et al. 2000). Their findings also indicate relatively high levels of overall genetic diversity within Borneo (also observed in our study in the Kinabatangan area, see Goossens et al. [2005a]), and they suggest that the habitat fragmentation and degradation during the last three decades had limited influence on genetic variability. Kanthaswamy et al. also argue that, because the mtDNA diversity in Sumatra is higher than in Borneo, the Sumatran population retains much older matrilines, the Bornean orangutans (p.10) would descend from early Sumatran founders, and that the Borneo island was colonized by Sumatran orangutans, which is in agreement with the Muir et al. (2000) and Warren et al. (2001) studies, and confirmed by biogeographic evidence detailed in Harrison et al. (2006). Further, Kanthaswamy et al. explain the reduced Bornean mtDNA variability by founder effects associated with relatively recent colonization of the island followed by geographical expansion, genetic drift and bottlenecks of specific lineages caused by habitat reduction.
1.5.1.5 Small scale: Genetic diversity in a fragmented population of Bornean orangutan (Goossens et al. 2005a) and rivers influence the population genetic structure of orangutans (Jalil et al. 2008)

In these papers, the authors investigated the genetic structure within and among forest fragments scattered alongside the Kinabatangan river, in Sabah, Malaysia, using microsatellites (Goossens et al. 2005a) and mitochondrial DNA (Jalil et al. 2008). A large number of samples (279), shed hair in night nests (Goossens et al. 2004) and feces, were collected during boat surveys on the river and ground surveys (Ancrenaz et al. 2004b). Using 14 human-derived microsatellite loci, 200 different individuals were genotyped and it was found that genetic diversity was high ($H_E = 0.74$), as confirmed by the Kanthaswamy et al. (2006) study, and that genetic differentiation was significant but not very high between the lots ($\text{average } F_{ST} = 0.04$, $p < 0.001$) with $F_{ST}$ values ranging from low (0.01) to moderately large (0.12) values (Goossens et al. 2005a). The role of the river as a natural barrier to gene flow was demonstrated by finding significantly higher pairwise $F_{ST}$ values across the Kinabatangan river than between forest patches from the same side of the river (Goossens et al. 2005a). This was confirmed by Jalil et al.’s (2008) data when 73 individuals (out of the 200 identified by Goossens et al. [2005a]) were sequenced for the control region of the mitochondrial DNA. Orangutan samples on each side of the Kinabatangan river were significantly differentiated by a high value of molecular variance ($F_{ST} = 0.404$, $p < 0.000$) (Jalil et al. 2008), confirming previous studies that differentiation is much higher in mtDNA than in microsatellite data, and as expected due to its much lower $N_e$. 
1.5.1.6 Genetic signature of anthropogenic population collapse in a Bornean orangutan population (Goossens et al. 2006a)

For this study, the same samples were used as Goossens et al. (2005a), but the authors addressed a very different question. The aim was to determine whether there was a signal for a population decline, and whether such decline could be quantified and dated. Indeed, the high level of genetic diversity found in orangutans by Goossens et al. (2005a) and Kanthaswamy et al. (2006) does not necessarily indicate that habitat fragmentation has not had a strong impact on patterns of genetic diversity, contrary to the conclusion of Kanthaswamy et al. (2006). The reason for this is that when a population size drops, genetic diversity as measured by $H_e$ is little affected, particularly when the time since the population collapse is small and the original population was large. Different approaches were used by these authors, including summary-statistics-based and Bayesian full-likelihood methods (see above). The first approach allowed them to demonstrate that there was a strong signal for a population bottleneck, and that it was independent of the mutation models that were assumed. The second approach allowed them to make inferences under two classes of demographic models (Beaumont 1999). Under both demographic models the authors detected a major population decrease and found no support for growing or even stable populations. Orangutan populations in the sampled area appeared to have declined by at least a factor of 50 (with 95% probability) or 100 (with 90% probability). The third approach was an extension of the second approach (Storz and Beaumont 2002). Under this model it was possible to estimate the present-day and ancient effective population size and to date the start of the population collapse. The conclusion was that the collapse of the orangutan populations in the Kinabatangan area most probably started in the last few centuries, perhaps in recent decades. It should be said that the posterior distribution for $T$ (the time since the population change) was quite wide. However, with nearly no support for dates older than $\sim$2000 years, it allowed the authors to exclude ancient events such as climatic changes, the arrival of hunter-gatherers or farmers as a cause for the signal of population decrease. With a median around $T = 210$ years, which coincides well with the start of forest exploitation in Sabah, these data strongly suggested for a major impact of habitat degradation in decreasing genetic diversity in orangutans see Fig. 1.1). The
good news of these studies, though, for the orangutans in the Kinabatangan, and most probably in the whole state of Sabah is that the populations still exhibit high levels of genetic diversity, but that immediate steps need to be taken to reconnect remnant forest patches, halt further deforestation and envisage managed translocations. Finally, genetic diversity estimates, as in previous studies used individuals of different ages, and hence different generations. Thus, from that point of view, genetic diversity might be overestimated.

1.5.1.7 General discussion on population genetics of Bornean orangutan

These studies show that our knowledge of the population genetics of orangutans is still at a very preliminary stage. Indeed, a better understanding of the patterns of genetic diversity requires many natural populations to be sampled. However, most studies have typically used individuals from rehabilitation centres. When large-scale studies are performed this is not necessarily problematic, if we can assume that the individual's geographic origin can be at least approximately determined. Unfortunately, some studies indicate that there are a number of serious problems with this issue. For instance, Warren et al. (2000) mentioned that the orangutans originating from West Kalimantan and Central Kalimantan were transferred to Wanariset Orangutan Reintroduction Project, in East Kalimantan, and released into forests containing no wild populations of orangutans. This would mean that Bornean orangutans from different subspecies (P. p. pygmaeus, P. p. wurmbii, P. p. morio) were mixed in the forests of East Kalimantan. Similarly, Kanthaswamy and Smith (2002) noted that the exact origin of the animals
they analyzed was unknown, and that translocation of Sumatran individuals could explain some of their results. While it is difficult at this stage to determine the impact of such translocations, it is clear that they represent a real problem for orangutan conservation programs and for studies aiming to identify the population subdivision and the amount of gene flow between wild orangutans. Even limiting ourselves to the studies for which the geographical locations were ascertained, some inconsistencies appeared in the amount of genetic differentiation estimated. Goossens et al. (2005a) found that even at relatively small scale, some differentiation was measurable, whereas Warren et al. (2000) found little differentiation at much larger scales. This stresses the fact that genetic studies should use samples with an exact location of origin, and avoid as much as possible samples coming from rehabilitation centers. More should be done to increase the geographic sampling.

Figure 1.1 Time since the population collapse (from Goossens et al. 2006a). The posterior distribution for the time since the population collapse is represented on a logarithmic scale. These distributions have a median around 210 years. Most of their mass is concentrated in recent years with a sharp decrease as time goes back. Indeed, 10, 20, 50, 80, and 90% of the distribution mass are below 10, 35, 210, 950, and 1900 years, respectively. The thin and thick lines correspond to S1 and S2, respectively. The prior shown as a dashed line, its median being 100,000 years ago. The vertical dashed line corresponds to the 95% quantile of the posterior distribution. Arrows correspond to the dates of arrival of the first hunter-gatherers (HG) or farmers (F), or to the start of the forest exploitation (FE).
and using the same loci across studies. One interesting and consistent result was that rivers appear to play a significant role at different geographic and hence temporal scales. The fact that Jalil et al. find very high levels of genetic differentiation using mtDNA data using a subsample of Goossens et al.’s (2005a) data support the idea that mtDNA may be influenced by events much older than those that shaped microsatellite diversity. Indeed, whereas Goossens et al. (2006a) showed that recent habitat fragmentation significantly affected the genetic make-up of orangutans from the Kinabatangan, mtDNA patterns appear to be more affected by events related to the colonization of the island.

It is believed that the orangutans started to colonize the island of Borneo from the south-east corner of Sumatra, and that their dispersal in Borneo was probably determined by geographical barriers such as mountain ranges and large rivers. Jalil et al.’s mtDNA data suggest a possible two-route colonization of the Kinabatangan river, from Mount Kinabalu refugia, which supports the hypothesis that dispersal occurred along the foothills of central mountain ranges that acted to geographically isolate populations. More testing of phylogeographic hypotheses is, however, needed. Another problem that would need to be taken into account is the fact that population structure can produce similar signals as some demographic events, as noted by Goossens et al. (2006a). This point was stressed by Fischer et al. (2006) who also found a signal for population decline in their orangutan data, but concluded that their sampling scheme favored population structure as a more likely explanation. Similarly, the past demography of orangutans needs to be studied using well-identified samples.

1.5.2 Comparison with other great apes

Population genetic studies are more common in African great apes (gorillas, chimpanzees and bonobos) than in Asian great apes (Sumatran and Bornean orangutans). This difference is probably due to the fact that the three African great apes are social species, living in small to large groups, and spend more of their time on the ground. Therefore scientists have access to larger sample sizes and better quality samples. Orangutans are semi-solitary, very elusive and shy and almost exclusively arboreal. Access to large sample sizes is extremely difficult and samples such as feces are usually dropped from 20 to 30 m above the ground, decreasing their quality.
As for orangutans, it has been demonstrated that large rivers can present barriers to dispersal and therefore to gene flow for several other primate species (see Colyn et al. 1991; Ayers and Clutton-Brock 1992; Telfer et al. 2003), including (and above all) other great apes (Schwarz 1934; Gonder et al. 1997; Eriksson et al. 2004). To illustrate this, we can take the distribution of bonobos and chimpanzees in Africa. Indeed, *Pan paniscus* occurs only south of the Congo River while the four Pan troglodytes subspecies can be found north of this river, in a discontinuous distribution from west to east Africa: *P. t. schweinfurthii* ranges north of the Congo River and east of the Ubangui River, while *P. t. troglodytes* ranges west of the Ubangui River and east of the Niger River, and *P. t. verus* is found west of the Niger River to as far west as the Gambia River (Schwarz 1934). Finally, *P. t. vellerosus*, also called the Nigerian chimpanzee, could be separated from the central chimpanzee by the Sanaga River, rather than the previously assumed Niger River (Gonder et al. 1997). Gonder et al.’s (1997) study showed the importance of appropriate sampling if it is to show the role of rivers as boundaries to gene flow.

For the Bornean orangutan, as shown above, rivers and mountains form the main barriers between the three subspecies described: *Pongo pygmaeus pygmaeus*, the north-west Bornean orangutan, occurs in West Kalimantan and Sarawak, with natural boundaries being the Rajang and Kapuas rivers; *P. pygmaeus wurmbii*, the central Bornean orangutan, is found in Central Kalimantan, with natural boundaries being the Kapuas and Mahakam rivers; and finally *P. pygmaeus morio*, the north-east Bornean orangutan, ranges in East Kalimantan and Sabah, from Mahakam and Rajang rivers. Warren et al. (2001) were more cautious with the subspecies ‘dilemma’ and described four distinct subpopulations in Borneo, using mitochondrial DNA control region sequences from orangutans sampled on the island: (1) South-west and Central Kalimantan, (2) North-west Kalimantan and Sarawak, (3) Sabah, and (4) East Kalimantan. If we compare the distribution of these populations with the distribution of the three subspecies, it appears that *P. pygmaeus morio* could be split in two differentiated subpopulations (see a comprehensive map of that distribution in Caldecott and Miles [2005]). Jalil et al.’s (2008) results confirm the differentiation between Sabah and East
Kalimantan orangutans. Large sampling in populations located between the Kinabatangan river and the Kayan river, and between the Kayan river and the Mahakam river must be carried out to understand the biogeographic history of the orangutan in Sabah and East Kalimantan.

Paternity and relatedness studies are also rare in orangutans (only two studies so far: Utami et al. [2002], Goossens et al. [2006b]), probably for the same reasons mentioned above: semi-solitary life, exclusively arboreal and difficult access to samples. On the other hand, this is not the case for chimpanzees where genetic studies on paternity and relatedness are numerous (see Morin et al. 1994; Gagneux et al. 1997, 1999a; Constable et al. 2001; Vigilant et al. 2001), and in a lesser extent for bonobos (see Gerloff et al. 1999) and gorillas (see Bradley et al. 2005).

Overall genetic studies on wild orangutan populations are rare. Orangutans are solitary, elusive animals and therefore extremely difficult to sample. The only comprehensive population genetic study using samples collected in the wild was carried out along a large river, increasing the chance of sighting wild orangutans and fresh nests. Sampling of wild orangutans in large primary (or even secondary) forest represents a formidable challenge and requires manpower. Nevertheless, if we want to understand population genetic structure, fill the gaps of current Bornean phylogeographic data (e.g. Warren et al. 2001; Steiper 2006; Jalil et al. 2008), as well as identify the parameters which shape the genetic variability and structure in the Bornean and Sumatran orangutans, we will need to carry out large sampling in wild populations in both the Borneo and Sumatra islands. During the 2006 International Primatological Society conference held in Entebbe, Uganda, a network was set up by population geneticists and evolutionary ecologists B. Goossens and M.W. Bruford (Cardiff University), L. Chikhi (CNRS, Toulouse University) and M. Krützen and C. van Schaik (Institute of Anthropology, Zürich) with the aim of developing a sustained collaboration on orangutan phylogeography, sociogenetics, conservation genetics and functional genomics, and to share expertise and samples, in order to improve our understanding of the species’ biology and our ability to implement effective conservation measures.
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