DETERMINATION OF ORANGUTAN SUBSPECIES USING GENETIC TECHNIQUES

Leona Chemnick, Yunwu Zhang, and Oliver Ryder
Zoological Society of San Diego
Center for Reproduction of Endangered Species

When the Orangutan Species Survival Plan (SSP©) was formed in the early 1980s, determining the subspecies status of orangutans was crucial to the demographic and genetic management of the captive population. A major goal of the SSP©, appropriate gene pool management, could not be accomplished without a clear policy regarding subspecies identification in the context of an overall plan for handling the species in captivity.

Problems arose in making a determination of subspecies relying solely on physical differences. While morphological characteristics between the Bornean orangutan (*Pongo pygmaeus abelii*) and the Sumatran orangutan (*Pongo pygmaeus pygmaeus*) could be rather clear, individual variation among some wild-born orangutans complicated definitive subspecies determination, especially in younger or female animals. However, zoos often had only morphological differences to rely on since exact capture sites were unknown or records were incomplete.

There were also some concerns about the validity of subspecies designations and their significance for conservation and population management, so it was decided that the first step in proper management would be to collect genetic information on the founder animals. This information could then be used to review studbook data, and for the eventual genetic management of captive orangutans in SSP© collections.

Cytogenetic techniques were initially selected for differentiating between the orangutan lineages. We had available as a karyotypic marker (Seuanez et al. 1979; Seuanez 1982) a small pericentric inversion that occurred on the second chromosome pair, most notable in G-banded karyotypes (Figure 1). The chromosome 2 pair in the Sumatran orangutan have longer upper or small arms, and the Bornean chromosome 2 pair have shorter small arms. If a cut is made near the centromere of one chromosome type and the piece inverted, it resembles the other; that cytological landmark is the chromosomal indicator between the two orangutan populations. Orangutans of mixed subspecies origin would have one of each chromosome 2 type, but the chromosomes of offspring of hybrids could resemble those of wild born (e.g. "pure" subspecies) animals. All other G-banded chromosome pairs except one look the same in both orangutans; an inversion does occur occasionally in the ninth chromosome, but it is not subspecies specific.

In 1982, the chromosomal studies on Bornean and Sumatran orangutans began in our Genetics department on all wild-born individuals in North America. Eventually samples arrived from 39 zoos in 21 states, Washington D. C., Canada, and Mexico. In cases where one or both wild-caught parents were not available for examination, we performed chromosomal analysis on all their first generation captive-born offspring.

The karyotyping project was basically completed by 1990, although we have continued to receive cases. To date we have analyzed a total of 151 animals (Figure 2), the most extensive samplings ever done on the two named subspecies. This number includes 60 wild-born individuals, two presumed wild-born animals, and 49 offspring of wild-born parents not available for sampling. Twenty-eight of the individuals we karyotyped, including six wild-born founders, had incorrect subspecies designations in the studbook. Among the founder animals, we suspect this was due to the problems associated with trying to determine subspecies based strictly on morphological characteristics. Four wild-born females and one wild-born male were initially reported as Bornean, and one wild-born female was first misidentified as Sumatran. All 28 orangutans had their designations corrected in the studbook based upon our results.

All the wild-born and presumed wild-born orangutans we analyzed were homozygous for the characteristic inversion on chromosome 2, and the only heterozygotes seen were in captive-born individuals of mixed subspecies ancestry. When we add in the results obtained by de Boer and Seuanez (1982; Figure 2) the total numbers are compelling. The inversion heterozygotes appear to be due to matings between individuals of the two subspecies in captivity. Based upon the populations sampled in the cytogenetic survey of SSP© orangutans, there are two genetically discrete orangutan populations fixed for alternative forms of the chromosome 2 inversion.

The fixed differences observed in chromosomal make-up are indicative of a lack of gene flow due to reproductive isolation (White 1973). However, chromosomal analyses can not provide accurate estimates of overall genetic distance, indicating how long ago the surviving populations may have diverged from a common ancestor. Also, subspecies identification on orangutans of unknown origin using just karyotyping can not predict with any certainty from which population their parents came. These questions were best determined through molecular genetic investigations.

To examine genetic divergence, we initially analyzed mitochondrial DNA (mtDNA) restriction site patterns in 14 of the animals we had karyotyped. Our data resulted in an estimated sequence divergence of 3.4% between the two orangutan chromosomal types, indicating a separation from a common ancestor of approximately one and a half million years ago (Ryder and Chemnick 1993).

More recently we studied a much larger number of founder orangutans, and sequenced the ND5 gene of mtDNA in 44 individuals, 21 Bornean and 23 Sumatran (Zhang et al. 2001). All these animals have been characterized by karyotyping. The results indicated a separation between the Bornean and Sumatran orangutan of about 2.3 million years, which was in line with the findings from our earlier study above, and with a number of other independent assessments of divergence including Bruce and Ayala 1979; Ferris et al. 1981; Caccone and Powell 1989; Janczewski et al. 1990; Xu and Arnason 1996; and Zhi et al. 1996. All these studies have concluded that the two orangutan subspecies are genetically distinct at levels more consistent with a species level divergence, similar to that found between the bonobo and the chimpanzee.

Another important finding from Zhang et al., with regards to subspecies determination, was the analysis of microsatellite variation in the 44 orangutans. Twenty polymorphic

human microsatellite loci were examined, and 19 were found to be informative (Figure 3). One locus, D3S2459, was unique in that there were no shared alleles between the two subspecies found at this locus. Interestingly, this locus is from human chromosome 3, which is homologous to orangutan chromosome 2, and may be associated with the pericentric inversion. The 18 other loci had one or more alleles shared by the two subspecies, but they also had one or more alleles unique to one subspecies or the other.

These microsatellite loci will be very useful in helping to qualify the subspecies identity of an orangutan from an unknown origin or to identify possible back crosses. The informative microsatellites are also valuable since they can be used to determine paternity or maternity in questionable pedigrees.

The primary purpose of our genetic studies was to address concerns regarding the evolutionary validity of subspecies designations and their biological significance for conservation; the Orangutan SSP© needed a clear policy regarding identification as to lineage, and a rational plan for the handling of the species in captivity.

Although we were not the first group to employ either karyotyping or DNA analysis in studying orangutans, we provided an extensive sampling of the two named subspecies. Based upon the populations sampled in the survey of SSP© orangutans, our findings helped confirm the existence of two clearly distinct phylogenetic lineages, which correspond to the two populations characterized by their G-banded morphology on chromosome 2.

The chromosomal investigations have been very useful to the studbook keeper and SSP© Management Group in making numerous breeding and management decisions, and greatly assisted the SSP© with its goal of characterizing the captive population. Chromosomal comparisons remain an important aspect of genetic analysis in support of SSP© programs and professional management of threatened and endangered species.

However, it is important to realize that we can also rely on molecular means for subspecies determination in orangutans. This is crucial when there is only a very small sample, when only non-invasively collected material such as hair or feces is available, or when a subspecies designation is required for an individual for which very little information is available.

We did the initial cytogenetic characterization of the orangutans when most of the wild-born individuals were still alive. The molecular work of Zhang et al. not only extended the results from the study we did initially, but more importantly, the microsatellite analysis can help reduce incidences of unsubstantiated subspecies in the orangutans remaining to be examined, as well as be used for parentage determination.

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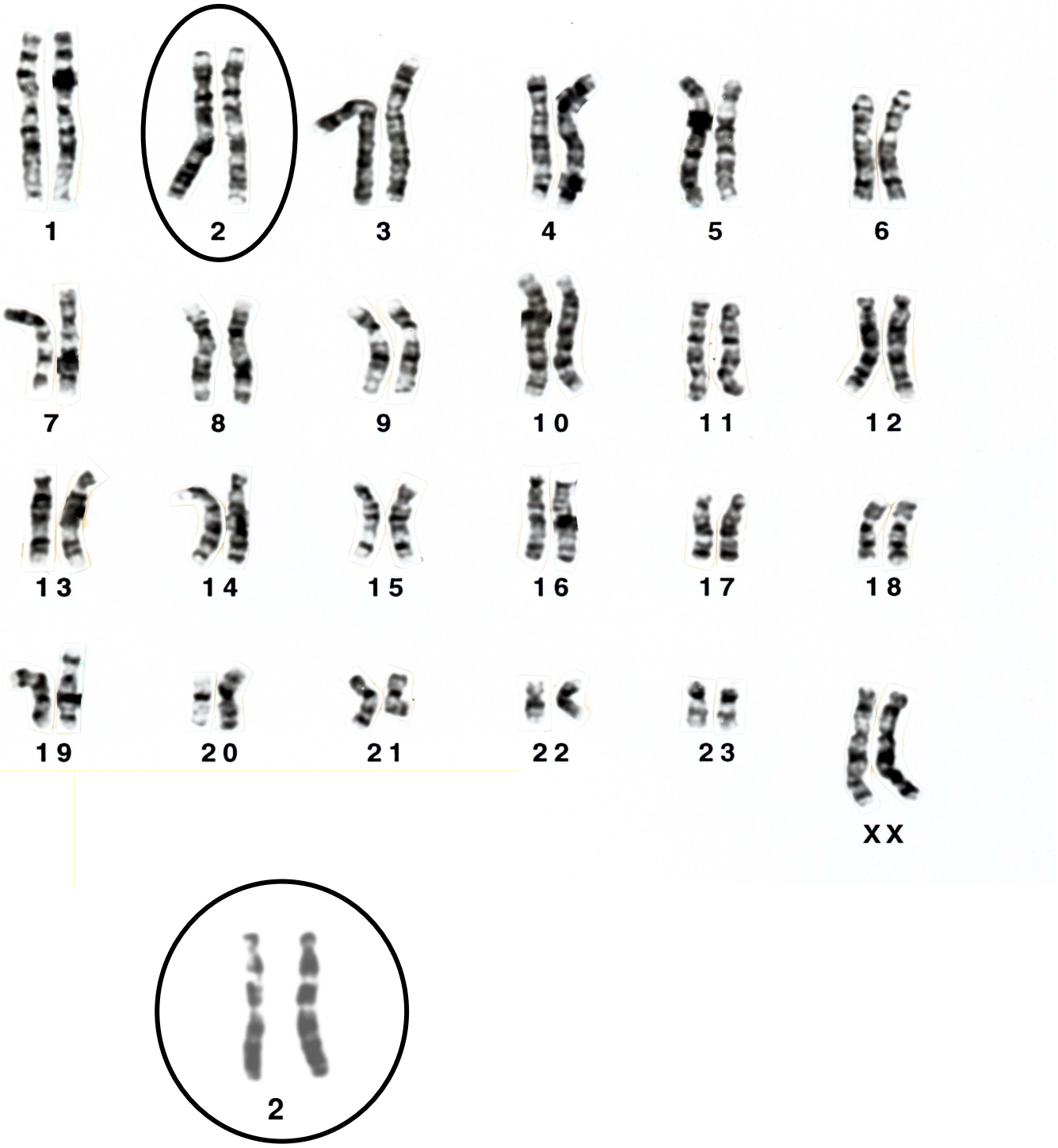
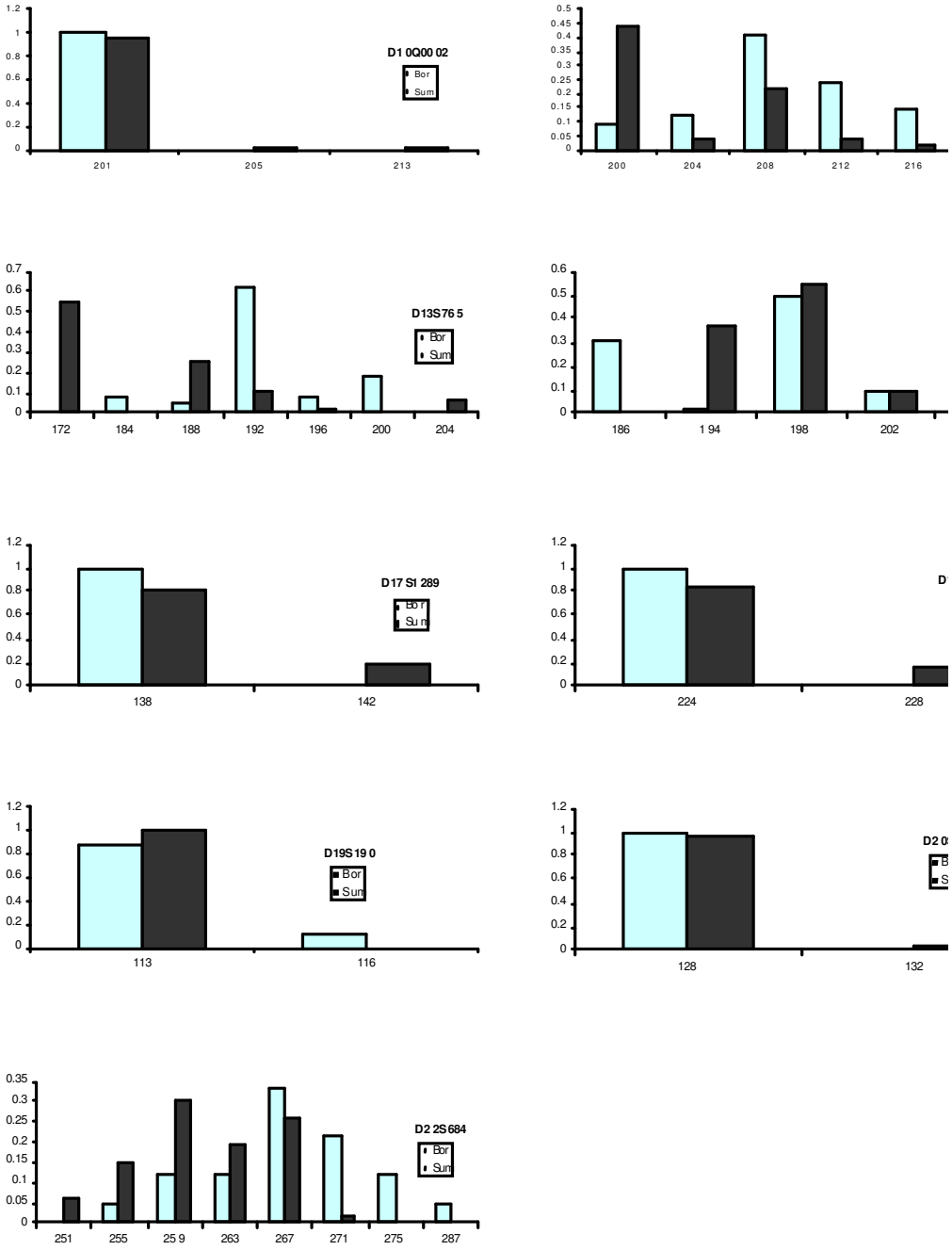


Karyotype of a Female Sumatran Orangutan with the Chromosome 2 pair circled, and a Bornean Chromosome 2 pair, circled below.

Figure 1

SUMMARY OF CHROMOSOME STUDIES	
151 ORANGUTANS KARYOTYPED (by SAN DIEGO ZOO)	**223 TOTAL ORANGUTANS KARYOTYPED (including de Boer & Seuanez study)**
60 WILD BORN (wb)	**94 WILD BORN (wb)**
28 BORNEAN	**51 BORNEAN**
32 SUMATRAN	**43 SUMATRAN**
0 HYBRID	**0 HYBRID**
89 CAPTIVE BORN	**127 CAPTIVE BORN**
27 BORNEAN	**39 BORNEAN**
29 SUMATRAN	**43 SUMATRAN**
33 HYBRID	**45 HYBRID**
2 UNKNOWN (presumed wb)	**2 UNKNOWN (presumed wb)**
2 BORNEAN	**2 BORNEAN**

Figure 2



Polymorphic Human Microsatellite Loci in Orangutans
The light bars represent the alleles found in the Bornean orangutans, and the dark bars are the alleles seen in the Sumatran individuals.

Figure 3