Population Subdivision and Gene Flow among Wild Orangutans

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ABSTRACT. Genetic variability among populations of orangutans from Borneo and Sumatra was assessed using seven SSR loci. Most SSR loci were highly polymorphic and their allele frequencies exhibited substantial variation across subpopulations. While significant genetic subdivision was observed among the island populations, genetic distance did not increase with geographic distance and sufficient gene flow persists to prevent marked genetic subdivision. Since it is unlikely that the Bornean Orangutans dispersed naturally among locations separated by such formidable geographic barriers, human assistance might already have altered their genetic structure. Our data suggests that there may be at least two subspecific clades of orangutans within Borneo while Central Kalimantan animals may have become more genetically related to animals in Sumatra due to human intervention.

Key Words: *Pongo pygmaeus*; Microsatellite polymorphism; Migration; Conservation.

INTRODUCTION

Preserving the functional and historical integrity of populations over the long term will increasingly demand informed intervention (GRUMBINE, 1994). Future interventions will not be efficient if managers ignore impacts of human activities on natural populations. Population history can now be at least partially but quantitatively reconstructed from genealogically informative data, such as the cross-species simple sequence repeat (SSR) [or microsatellite or short tandem repeat, STR] markers. As a consequence, historical reconstructions of both natural and human influence on many species for which insights are in greatest demand are now possible.

Several general phenotypic characteristics such as body size and weight, texture and coloration of hair, the shapes and sizes of facial flanges and throat sacs have been examined to determine the range of taxonomic differentiation between and among Sumatran and Bornean subpopulations (MACKINNON, 1974; RIJKSEN, 1978; WINKLER, 1988; COURTENAY et al., 1988; RIJKSEN & MEIJAARD, 1999), however, a systematic examination and a quantitative analysis of geographically different phenotypes within and between the two island subpopulations are still lacking. Nonetheless, primatologists generally agree that the *Pongo pygmaeus pygmaeus* in Borneo and *P. p. abelii* in Sumatra are two separate subspecies. Data from skull morphometrics, however, reveal significant distinctions among Sumatran, Southwestern Bornean and the remaining Bornean subpopulations (GROVES et al., 1992) suggesting that at least three genetically isolated orangutan populations exist in Borneo and Sumatra (their study did not include animals from Central or East Kalimantan). Furthermore, UCHIDA (1998) reported a difference in postcanine cusp morphology between Northwest and Southwest Kalimantan animals that was as large as those observed between Bornean and Sumatran populations.

Previous studies of different orangutan samples have reported considerable variability both

in DNA and protein coding loci (BRUCE & AYALA, 1979; KANTHASWAMY et al., 2001, in press; JANCZEWSKI, 1989; JANCZEWSKI et al., 1990; RYDER & CHEMNICK, 1993; MUIR et al., 2000; RUVOLO et al., 1994; WARREN et al., 2000, 2001; XU & ARNASON, 1996; ZHI et al., 1996). Despite these voluminous amounts of molecular data, information concerning genetic differentiation in orangutans at the subpopulation levels is still lacking because few investigators have studied a consistent panel of genetic markers across generations or geographic locations, and few highly polymorphic but quantifiable markers have been developed for such comparative studies (WARREN et al., 2000; KANTHASWAMY et al., 2001, in press). In the present study, as well as in that of KANTHASWAMY et al. (2001) and WARREN et al. (2000), the analysis of the distribution of the nuclear variation within and between the island populations suggest that Bornean Orangutans exhibit substantial genetic substructuring at several hierarchial levels.

While sister-species share many nuclear alleles, they differ significantly in the frequencies of some of these alleles (MORITZ, 1994). Genetic distances between congeneric species are generally greater than those between subspecies (AVISE, 1994). Even among conspecific local populations or demes within which an assortment of alleles is shared, allele frequencies can vary greatly (BRUCE & AYALA, 1979; MELNICK & HOELZER, 1992; PAETKAU et al., 1995; ST. GEORGE et al., 1998), as expressed by values of genetic distance between such demes (NEI, 1987). As a consequence, more genotypic information is required to identify the mechanisms that cause variation at the subspecific and subpopulation levels.

The orangutan home range is now restricted to the shrinking forests of Borneo and Sumatra. Triggered by a decline in numbers due to habitat fragmentation and poaching, wild orangutan populations are vulnerable to the loss of genetic variability and/or extinction (GALDIKAS, B., pers. comm.). While no information is available on the status of their effective population size (N_e), recent surveys have led to speculation that their population size is declining dramatically (FERBER, 2000), underscoring the need for the immediate development of alternative conservation models, which include genetic management and sufficient studies to monitor patterns of genetic heterogeneity and gene flow.

The total orangutan census size in Borneo is probably between 5,000 to 15,000 individuals while the Sumatran population is expected to include ca. 5,000 individuals (GALDIKAS, B., pers. comm.; FERBER, 2000). The Bornean population is represented by a network of geographically dispersed, and sometimes isolated subpopulations, each comprising between 20 and 30 individuals (BOSI, E., pers. comm.) arranged in groups of independent units. These units may include single individuals or aggregations of pairs of adult males, pairs of adult females with single infants, mother-offspring units and a juvenile male or adult female-juvenile male pairs (RODMAN, 1978). Groups are formed when these independent units congregate around food sources when food accessibility is unevenly distributed, or when individuals coordinate travel between food sources. Orangutan densities have been reported to be between ~ 4.0 per km^2 and ~ 7.0 per km^2 in East Kalimantan (RODMAN, 1978) and the estimated home range of Sumatran females and males vary between 700 and 900 ha and between 1,000 and 4,000 ha, respectively (SUGARDJITO et al., 1987; TE BOECKHORST et al., 1990). These estimates are consistent with the report of BOSI et al. (in press), who counted up to 7.0 nests per km^2 in the state of Sabah, North Borneo. A similar distribution and density of population can be assumed for Sarawak. While the Kapuas River acts as a natural obstruction to gene flow between animals in Western and Southwestern Borneo, the central mountain range (i.e. the Crocker Range) impedes dispersal of animals between both these populations and those in North and East Borneo. It has been estimated that the Bornean and Sumatran populations have been geographically separated since the Late Pleistocene, i.e. for ca. 10,000 years (COURTENAY et al., 1988).

We employed seven highly polymorphic SSR loci, originally cloned from the human genome, as markers for studies of genetic variation within and between orangutan populations in Borneo and Sumatra, and to estimate gene flow among several subpopulations in Borneo. We studied samples from 92 individuals from five distinct populations, including one from Northern Sumatra. The Bornean populations were from Sabah, Sarawak, Central Kalimantan, and Northeast Kalimantan.

MATERIALS AND METHODS

Only samples from adults with no known relationship to other sampled animals were used in this study. Subjects included both Sumatran (n = 19) and Bornean (n = 73) Orangutans; all samples were from wild captured or zoo animals that were originally derived from the wild. All 19 Sumatran individuals represent populations from Ketambeh and Suak Balimbing in Northern Sumatra. Sample sizes of Bornean Orangutans from Central Kalimantan (West and South Central Kalimantan), Sarawak, Kutai (Northeast Kalimantan), and East Sabah were 19, 14, 13, and 27, respectively (see Fig. 1 in KANTHASWAMY et al., 2001). Samples from adjacent areas were combined to increase sample sizes when these locations were within: (1) the migratory range of male orangutans in Borneo [~ 50 km (GALDIKAS, B., pers. comm.)]; (2) a conceivable distance for gene flow as reported in chimpanzee (*Pan troglodytes*) [~ 400 km (MORIN et al., 1994)]; (3) the range for animal translocation by local Forest Departments (BOSI, E., pers. comm.). In addition, a homogeneity χ^2 test (see below) was used to determine whether or not genotype frequencies among samples from adjacent areas were sufficiently uniform to warrant pooling their data.

DNA was purified from blood, tissue or hair samples, or obtained in purified form from the Center for Reproduction of Endangered Species (CRES), Zoological Society of San Diego, California, U. S. A., the Laboratory of Genomic Diversity, National Cancer Institute, Frederick, Maryland, U. S. A., zoos in the U. S. A. that participate in the Species Survival Program (Auduborn Zoo, New Orleans, Louisiana; Birmingham Zoo, Alabama Zoological Society, Birmingham, Alabama; Cheyene Mountain Zoo, Colorado Springs, Colorado; Brookfield Zoo, Chicago Zoological Society, Brookfield, Illinois; Marine World U. S. A., Vallejo, California; Metro Zoo, Miami, Florida; San Francisco Zoological Garden, San Francisco, California; St. Louis Zoo, Woodland Park Zoological Gardens, Seattle, Washington; Yerkes Primate Center, Emory University, Atlanta, Georgia) and from captive as well as wild orangutan populations in Malaysia. Samples were shipped on dry ice to the Molecular Anthropology Laboratory, Department of Anthropology, University of California, Davis, California, U. S. A., where DNA was extracted from blood and tissue using the QIAGEN blood and tissue kits (QIAGEN, Inc., California, U. S. A.). We extracted up to 5 to 8 μg of DNA/200 μl blood or homogenized tissue, up to 10 ng/200 μl of DNA/shed hair, and up to 200 ng/200 μl of DNA/plucked hair. Some of our DNA extracts are from shed hairs that were more than 6 months old.

We used human primers that had successfully amplified human homologs in rhesus monkey (KANTHASWAMY & SMITH, 1998) and baboon (SMITH et al., 1999) genomes to characterize genetic variation in orangutans. SSR markers were amplified enzymatically by PCR and analyzed for size polymorphisms. Specific methods of amplification, polyacrylamide gel electrophoresis, and digitalized gel imaging are described in detail elsewhere (MORIN & WOODRUFF, 1992; MORIN & SMITH, 1995; MORIN et al., 1997; KANTHASWAMY & SMITH, 1998; KANTHASWAMY et al., 2001, in press).

To evaluate the effectiveness of our panel of primers in distinguishing the genotypes unique to, or more typical of, Bornean Orangutans from those unique to, or more typical of, Sumatran Orangutans, we used a computational program described by PAETKAU et al. (1995, 1997). This program, based on an assignment index, calculates the expected frequencies of individuals' genotypes in the Bornean and Sumatran populations, respectively, and determines the relative probabilities of an orangutan's genotypes at all loci appearing in each of the two geographic populations.

Biosys-1 program release 1.7 computer program (SWOFFORD & SELANDER, 1981) was used to estimate allele and genotype frequencies and calculate observed heterozygosity (H_I) and gene diversity, or the expected level of heterozygosity under genetic equilibrium conditions (H_S: NEI, 1987). Expected genotypes under random mating were computed using the methods described by LEVENE (1949) and unbiased values of H_S were estimated using his correction formula. Agreement between H_I and H_S for each locus was examined using a χ^2 goodness of fit test (HARTL & CLARK, 1989); although this procedure does not test explicity for Hardy-Weinberg Equilibrium (HWE), it detects the presence of null alleles (CALLEN et al., 1993). In our lab, we confirm the presence of a null allele when an individual who is apparently homozygous for a particular allele exhibits a single amplified fragment that does not stain as intensely as bands of that allele in true homozygotes [when quantified with MspI digested pBR322 molecular weight markers (New England Biolabs, Massachusetts, U. S. A.)].

We used the GENEPOP computer program (RAYMOND & ROUSSET, 1995a) to detect departures from HWE by estimating the inbreeding coefficient (F) described by WEIR (1990a, b), consistently positive and negative values of which, across loci, indicate a deficiency and excess of heterozygosity, respectively. The Markov chain method, which gives the exact distribution for allele frequencies in small populations (NEI, 1987), was used to correct for stochastic change of allele frequencies. Additionally, because F can be disproportionately influenced by rare alleles, we tested the significance of the average F across all loci using the method of ROBERTSON and HILL (1984). A contingency χ^2 test was used to determine if allele and genotype distributions were homogeneous across subpopulations of sufficient sample size and Fisher's exact probability test (or Fisher's exact test, see RAYMOND & ROUSSET, 1995b) was performed in all other cases.

Using GENEPOP, WRIGHT's Fixation Indices (F_{st}, F_{is}, and F_{it}, 1943, 1951) were modified to examine the orangutan population genetic structure based on procedures described by COCKERHAM (1973) and WEIR and COCKERHAM (1984); these procedures included a "weighted" analysis of variance of F_{st} computed for our multilocus SSRs. These estimates were used to measure the extent to which these taxa have become genetically differentiated via geographic or reproductive isolation followed by inbreeding. It has been previously demonstrated that WEIR and COCKERHAM's (1984) pairwise F_{st} estimates of genetic differentiation among subpopulations based on SSR loci are highly correlated with estimates of genetic distance derived from mtDNA analysis (RICHARD & THORPE, 2001).

To ascertain the level of pairwise genetic differentiation between subpopulations, we used NEI's (1987) standard genetic distance (D). PAETKAU et al. (1997) showed that NEI's D performed particularly well in defining the genetic architecture of a population composed of geographically dispersed subpopulations when genetic drift is the primary force driving the genetic differentiation. The use of D in addition to F_{st} enabled us to compare our SSR data on orangutan interpopulation genetic variation with data from protein coding loci and VNTRs generated by others. SCRIBNER et al. (1994) showed that while estimates of genetic distance between regional populations based on allozyme, minisatellite, and SSR loci were generally in agreement, spatial estimates of variation among these populations, as measured using WRIGHT's F_{st}, were greater

for allozymes than for VNTRs (probably because the highter VNTR mutation rates diminished the influence of gene flow).

Gene flow was estimated by substituting the standardized estimate of genetic variation among populations (F_{st}) in equation: $N_eM = (1/F_{st} - 1)/4$, where N_e is the effective population size of the recipient subpopulation, and M is the rate of gene flow (WRIGHT, 1943). While this equation assumes the infinite island model of population structure and gene flow to which few populations strictly conform, F_{st} provides a useful approximation of the relative magnitude of gene flow in a subdivided population under equilibrium conditions (SLATKIN & BARTON, 1989). A variety of theoretical models indicate a robust relationship between F_{st} and the product of M and N_e (SLATKIN & BARTON, 1989). Gene flow rapidly lowers F_{st} relative to the elevation of F_{st} by genetic drift and eliminates the effect of inbreeding within subpopulations.

RESULTS

Our panel of SSR loci exhibited high values of gene diversity due to their relatively high actual and effective allele numbers (see Tables 1 & 2). While most alleles were shared among all demes, we observed substantial differences in the frequencies of specific alleles among geographic locales (p-value $<<<0.0001$; $d.f. = 14$; the per locus F_{st} also illustrates this variation, see below). Due to the regional differences in genotype frequencies, over 65% of the 19 Sumatran and 73 Bornean individuals were sorted into their correct island of origin (Sumatra vs Borneo) using PAETKAU et al.'s (1995, 1997) method.

Except for D5S1470, these loci also exhibited a high concordance between the values of expected (H_s) and observed (H_i) heterozygosities (Table 2), indicating an absence of null alleles. For D5S1470, the absence of silent alleles was confirmed empirically homozygotes at this locus

Table 1. Mean numbers of actual (n_a) and effective (n_e) number of alleles and the mean values of observed heterozygosity (H_i) and expected heterozygosity (H_s gene diversity) based on seven SSR loci.*

Population	Mean n_a	Mean n_e	Mean H_i	Mean H_s
Sumatra	6.4 ± 1.3	4.2 ± 1.5	0.62 ± 0.13	0.72 ± 0.15
Central Kalimantan, Borneo	7.1 ± 1.5	5.3 ± 1.5	0.78 ± 0.13	0.80 ± 0.06
Sarawak, Borneo	5.7 ± 1.5	3.8 ± 0.8	0.65 ± 0.17	0.72 ± 0.07
Northeast Kalimantan, Borneo	3.4 ± 2.1	2.7 ± 1.4	0.67 ± 0.42	0.49 ± 0.31
Sabah, Borneo	5.1 ± 1.3	3.3 ± 0.9	0.67 ± 0.27	0.65 ± 0.17

* Allele and genotype frequencies are available upon request.

Table 2. Observed (H_i) and expected (H_s or gene diversity) heterozygosities and actual (n_a) and effective (n_e) number of alleles for seven SSR loci.

Locus	H_i	H_s	χ^2; $df = 1$	n_a	n_e
D1S548	0.78	0.74	0.68 ($p > .05$)	8	3.90
D1S550	0.83	0.82	0.02 ($p > .05$)	9	5.35
D5S1457	0.64	0.74	5.23 ($p < .02$)	7	4.59
D5S1470	0.53	0.77	28.5 ($p < .0001$)	8	4.35
D6S501	0.78	0.81	0.45 ($p > .05$)	9	5.20
D12S67	0.78	0.84	1.61 ($p > .05$)	8	6.06
D19S255	0.52	0.48	0.53 ($p > .05$)	4	1.94
Mean	0.69 ± 0.13	0.74 ± 0.12	–	7.6 ± 1.7	4.5 ± 1.3
Mean*	0.72 ± 0.12	0.74 ± 0.13	–	7.5 ± 1.9	4.5 ± 1.5

*Estimates excluding D5S1470.

Table 3. Inbreeding coefficient (F) as a measure of heterozygote deficiency or excess.

Population	D1S548	D1S550	D5S1457	D5S1470	D6S501	D12S67	D19S255
Sumatra	0.138	0.186	0.023	0.498	0.285	−0.053	−0.165
Central Kalimantan, Borneo	0.061	0.003	0.294	−0.003	0.189	−0.043	−0.178
Sarawak, Borneo	−0.096	0.348	−0.092	0.119	0.490	0.383	−0.393
Northeast Kalimantan, Borneo	−0.500	−0.472	−0.268	−0.043	−0.472	−0.056	−0.043
Sabah, Borneo	−0.125	−0.228	0.307	0.363	−0.251	−0.060	−0.109

consistently yielded the appropriate concentrations of PCR product, suggesting that both homologs were amplified; additionally, the effective allele number at this locus was similar to the mean value for all loci). The value of H_I ranged from 0.52 to 0.83, with a mean value of 0.69 ± 0.13, while the value of H_S ranged from 0.48 to 0.84, with a mean value of 0.74 ± 0.12 (Table 2).

Genotype frequencies at several loci did not conform to HWE, as illustrated in Table 3 by the biased ranges of *F* values. Deficiencies in heterozygotes, indicated by positive *F* values, occurred for all but two loci in the Sumatran population. Most Bornean demes (excluding Northeast Kalimantan) exhibited *F* values that, while varying greatly among loci, also indicated homozygote excess. This is suggestive of a presence of systematic inbreeding within the demes, but it does not preclude any effects of gene flow, genetic drift or combinations of the two.

The Bornean demes exhibited moderate to large F_{st} (Table 4) for all loci suggesting that these subpopulations are highly genetically differentiated. Cross-loci estimates ranged from 0.06 to 0.23 with an average value of 0.11. While most F_{st} values were tightly clustered, somewhat surprisingly, the basic pattern of F_{st} estimates remained unchanged when the Sumatran population was included in the analysis (mean F_{st} = 0.11, range: 0.08 – 0.20), further illuminating the genetic substructuring in Bornean Orangutans. However, the exclusion of locus D5S1470 from this analysis reduced the mean F_{st} in Borneo to 0.09 (and the range of values to 0.06 – 0.14). The mean F_{is} and F_{it} in Borneo were −0.04 (range across seven loci: −0.26 – 0.12) and 0.07 (range: −0.09 – 0.32), respectively. With the inclusion of the Sumatran population, the analysis yielded a mean F_{is} of −0.003 (or effectively zero; range: −0.25 – 0.21) and a mean F_{it} of 0.10 (range: −0.10 – 0.37), suggesting that the primary cause of genetic differentiation among Bornean Orangutans is indeed random genetic drift.

Table 5 gives estimates of genetic distance and gene flow (the two are inversely correlated) between each pair of subpopulations in Borneo, based on WEIR and COCKERHAM's (1984) pairwise F_{st}. Our estimates of pairwise N_eM range from 1.1 to 9.3 per generation. When Central Kalimantan is eliminated from all comparisons, this range declines dramatically to 1.1 – 3.1 per

Table 4. Values of F_{st} and N_eM (based on F_{st}) of five orangutan subpopulations.

		F_{st}	
Locus	Borneo	Borneo & Sumatra	N_eM
D1S548	0.091	0.089	2.6
D1S550	0.084	0.090	2.5
D5S1457	0.091	0.092	2.5
D5S1470	0.234	0.199	1.0
D6S501	0.070	0.090	2.5
D12S67	0.061	0.081	2.9
D19S255	0.137	0.117	1.9
Mean	0.110 ± 0.06	0.108 ± 0.04	2.3 ± 0.63
Mean*	0.090 ± 0.03	0.093 ± 0.01	2.5 ± 0.33

*Estimates excluding D5S1470.

Table 5. N_eM matrix based on pairwise F_{st} estimates (below diagonal) and standard genetic distance (D) estimates (above diagonal).

Population	Sumatra	Central Kalimantan	Sarawak	Northeast Kalimantan	Sabah
Sumatra	–	0.167	0.411	0.496	0.294
		(0.158)	(0.392)	(0.477)	(0.279)
Central	10	–	0.216	0.611	0.403
Kalimantan	(9.2)		(0.220)	(0.487)	(0.388)
Sarawak	2.9	9.3	–	0.443	0.315
	(2.9)	(8.2)		(0.282)	(0.283)
Northeast	1.2	1.1	1.3	–	0.174
Kalimantan	(1.6)	(1.6)	(2.3)		(0.146)
Sabah	3.1	2.1	2.6	2.8	–
	(3.1)	(2.1)	(2.8)	(5.0)	

Estimates excluding D5S1470 are in parentheses.

generation, still sufficient to prevent substantial fixation or extinction of alleles through genetic drift (FALCONER, 1981). Genetic differentiation among the Bornean populations, based on D, point toward differences that are as high as differences observed between the Bornean and Sumatran Orangutans and that are uncorrelated with geographical distances or barriers.

Whatever the reason (e.g. sampling effects) for the discrepancy between the observed and expected heterozygosity values at D5S1470, the exclusion of this locus led to an approximately 20% decline in the estimates of diversity and slight increase in the estimate of gene flow.

DISCUSSION

Except for JANCZEWSKI (1989), JANCZEWSKI et al. (1990), ZHI et al. (1996), WARREN et al. (2000, 2001), MUIR et al. (2000), and KANTHASWAMY et al. (2001, in press) who used samples of Bornean Orangutans of known origin, past studies have been restricted to determining the extent of genetic differentiation among zoo-derived samples of Bornean and Sumatran Orangutans. Each of those studies, except MUIR et al. (2000), recommended the elevation of the taxonomic status of the Sumatran and Bornean Orangutans from subspecies to species. Based on the island-specific chromosome 2 pericentric inversion, RYDER and CHEMNICK proposed the reclassification of *P. p. pygmaeus* and *P. p. abelii* as two allopatric species even though captive animals produced fertile offspring and viable backcrosses to either taxon when interbred (see RYDER & CHEMNICK, 1993). Based on two complete mitochondrial (mt) DNA sequences, one each from Borneo and Sumatra, XU and ARNASON (1996) showed a ca. 8% divergence, while JANCZEWSKI (1989), JANCZEWSKI et al. (1990), and ZHI et al. (1996), based on allozymes, mtDNA RFLP, mt16sRNA sequences and nuclear minisatellites, reported considerable differentiation among subpopulations between and within the respective islands. Although ZHI et al. (1996) concluded that there was insufficient genetic differentiation among the Bornean animals to regard any subpopulation as separate subspecies; they estimated that *P. p. pygmaeus* and *P. p. abelii* diverged approximately 1.5 million years ago. MUIR et al. (2000), however, argued against a simple Sumatran-Bornean divergence despite showing that the Sumatran and Bornean animals generally (of the 30 composite sequences that were analyzed, only one Sumatran individual clustered with the Bornean animals) formed separate monophyletic clades; their study was based on concatenated mtND3 and cytochrome b sequences. They also showed that Bornean populations were far less diverged ($\theta = 0.4\%$) than Sumatran populations ($\theta = 9.0\%$).

Based on SSR analyses, KANTHASWAMY et al. (2001) and WARREN et al. (2000) provided evi-

dence of high levels of divergence among the Bornean Orangutans as a result of genetic drift. The effect of genetic drift is particularly obvious between the East and West Bornean demes (WARREN et al., 2000). WARREN et al. (2000), however, did not detect any differentiation between the East and West Kalimantan orangutans in contrast to our study's homogeneity test for these two locations. Based on the sequence analyses of fragment length homozygotes of the monomorphic vWF locus, KANTHASWAMY et al. (2001) showed greater levels of differentiation among the Bornean Orangutans than among different subspecies of chimpanzees suggesting dramatic subspeciation within Borneo. Both WARREN et al. (2001) and KANTHASWAMY et al. (2001) reported marked genetic differentiation between Bornean and Sumatran Orangutans, supporting the occurrence of allopatric speciation between the two island populations.

Despite ZHI et al.'s (1996) observation, which was based primarily on mtDNA sequence analysis, that there was a lack of substantial genetic differentiation among regional populations in Borneo, both KANTHASWAMY et al.'s (2001) and WARREN et al.'s (2000, 2001) conclusions agree with that of ALTHEIDE, T. K. (pers. comm.), whose analysis of several Y chromosome haplotypes showed higher levels of differentiation among Bornean Orangutans than among Sumatran Orangutans, chimpanzees or gorillas, respectively. WARREN et al. (2001) identified four distinct Bornean subpopulations based on mt control region sequences: Southwest and Central Kalimantan; Northwest Kalimantan and Sarawak; Sabah; East Kalimantan.

Our panel of SSR loci exhibits sufficiently high levels of genetically unique variation at the level of the individual to uniquely characterize any randomly chosen orangutan in a population as large as 29 billion and provide a parentage exclusion probability of over 0.99 (KANTHASWAMY et al., in press). These estimates are striking given the widely accepted worldwide orangutan census of ca. 20,000 (albeit relatedness among those in the census population renders both exclusion probabilities overestimates). The result of our assignment test, in agreement with the more traditional algorithms, indicated that genotypes for this panel of seven loci could effectively identify the island of origin of approximately two-thirds of the sample studied. As such, these loci should facilitate studies of orangutan population genetics and phylogenetics and provide more accurate information on gene flow and genetic substructuring among populations of *Pongo*.

Most of the alleles that were observed at all loci occur in all subpopulations. This sharing of alleles may be due to recent gene flow among these subpopulations due to habitat shrinkage, animal translocations or the retention of polymorphisms that predate the genetic separation of these subpopulations. Nevertheless, the observed allele frequencies are statistically significantly different among all populations at all seven loci. This interlocality variation has also been shown in other studies on primate nuclear loci. For instance, MELNICK and HOELZER (1992) reported that 9% of the total intraspecific diversity in wild rhesus macaque populations was attributable to variation among geographic units, an estimate similar to that for F_{st} for orangutan populations studied here (0.11).

Our results, as indicated by the N_eM, F_{st}, and D values, reveal substantial genetic differentiation among the Bornean subpopulations. Genetic differentiation among Bornean subpopulations is as high as that between the Sumatran and Bornean Orangutans (KANTHASWAMY et al., in press). This is consistent with WARREN et al.'s (2001) and KANTHASWAMY et al.'s (2001) report of higher levels of sequence divergence within the mt control region as well as fragment length homozygotes of the monomorphic vWF locus among Bornean Orangutan groups. The high F_{st} values suggest that genetic drift is the primary cause of genetic differentiation while gene flow maintains homogeneity among these subpopulations. Additionally, $F_{is} \sim -0.04$ may be considered as indicating random mating within subpopulations or some influence from disassortative mating among these subpopulations due to movement of animals. However, the F_{it} value, which

is the overall inbreeding coefficient of an individual, of 0.07 indicates a reduction in individual heterozygosity relative to the total population.

Our estimates of gene flow among the four Bornean demes were comparable to those of other animal species (SLATKIN, 1985). In theory, the average exchange of one individual per generation between subpopulations (ca. $N_eM = 1$; $F_{st} = 0.20$), irrespective of size of the subpopulations, should be marginally sufficient to prevent dramatic genetic differentiation by genetic drift alone (ALLENDORF, 1983). Values of N_eM that are as low as 2 give considerable scope for genetic divergence through genetic drift. Our gene flow estimates suggest an average of between two and three migrants every generation. Even under these circumstances, migration among the Bornean subpopulations is restrained; this is understood because when members are exchanged without restraint, $F_{st} = F_{is} = F_{it}$, an equivalence not prevailing in this study.

Our data suggest that these orangutan subpopulations are not as isolated genetically as previously suggested by KANTHASWAMY et al. (2001). However, estimates of gene flow reported here might be influenced by translocations of animals from captive or wild stock in attempts to rescue threatened wild populations (KAVANAGH et al., 1987; ANDAU et al., 1994). This interpretation is consistent with the absence of any correlation between gene flow and geographic distance, which would be expected if dispersal responsible for the observed levels of gene flow occurred naturally. In Sabah alone, 85 animals were earmarked for translocation (ANDAU et al., 1994). This human influence might be particularly instrumental in facilitating gene flow between Sumatra and Borneo populations. In fact, the highest estimate of gene flow detected in the present study is that between Sumatra and Central Kalimantan. While detailed records are not available, orangutans have been translocated within Sumatra, from Sumatra to Central Kalimantan, and between areas of pocketed orangutans within Central Kalimantan, as part of the restocking, reintroduction and introduction programs (i.e. translocation programs) in Indonesia (RIJKSEN, 1978). The high estimates of gene diversity for Central Kalimantan (Table 1) might reflect this admixture. Therefore, we do not agree with WARREN et al.'s (2001) conclusion that the orangutan populations have not undergone any serious bottlenecks or collapses in population sizes, especially if members from once genetically isolated and differentiating demes are moved between locals.

The estimates of gene flow reported in this study might be inflated because SSR loci, like most other neutral nuclear and mtDNA markers, reveal sufficiently high degrees of genetic variability within a subpopulation that allele distributions among subpopulations overlap. Alleles might be shared between populations due to homoplasy or the persistence of ancestral polymorphisms rather than gene flow. It is also possible that our assumption that estimates of N_e are homogenous among subpopulations is inaccurate and, therefore, that it is inappropriate to estimate gene flow using WRIGHT's (1943, 1951) method. Estimates of N_e for each subpopulation, based on field studies and parentage assessments within wild orangutan populations, would provide confirmation of our estimates of the extent of gene flow per generation.

THORPE (1982) recommended the use of information on NEI's D when the species status of a population is unresolved by criteria based on morphology and physiology and the extent of reproductive isolation. KAWAMOTO et al. (1982) estimated genetic distances between 0.02 and 0.10 for the geographically isolated subspecies/species of Sulawesi macaques. These values are lower than our estimates for the Bornean subpopulations (0.17 – 0.61) and those of KANTHASWAMY et al. (in press) for the genetic distance (0.20) between Bornean and Sumatran Orangutans. Contrary to the findings of WARREN et al.'s (2001), who reported no distinct clustering by any of the Bornean subpopulations with the Sumatran population, our data show that the Central Kalimantan animals are clearly more genetically related to the Sumatran Orangutans than to any other Bornean subpopulation. As such, our findings, while reflecting the influences

of translocation programs in Central Kalimantan, are in general agreement with that of GROVES et al. (1992) and UCHIDA (1998) that at least two genetically homogenous groups exist in Borneo; collectively, the data of GROVES et al. (1992) and UCHIDA (1998) suggest that the northern-eastern, western, and southwestern populations, respectively, form three separate subspecific groups.

The discrepancy in estimates of divergence among the Bornean animals between our results and those of ZHI et al. (1996) and WARREN et al. (2001) could at least in part be attributed to differences in sampling. Moreover, the heavy reliance of both ZHI et al. (1996) and WARREN et al. (2001) on the maternally inherited mtDNA might have also contributed to this discrepancy. Inferences on genetic differentiation that are primarily based on the mt genome, a single genetic locus, can reflect a different population history from that based on several independent loci. Dependence on a single genetic locus diminished power to detect significant spatial or temporal structure of populations (SLATKIN & MADDISON, 1989, 1990). This is because genetic drift in populations entails random fluctuations in gene frequencies and these changes will follow different trajectories at independent loci (HARTL & CLARK, 1989). Although mtDNA analysis has its strength in describing the influence of maternal lineages on population structures, especially in female philopatric genus such as *Pongo*, it can underestimate gene flow among populations (see PALUMBI & BAKER, 1994). Conversely, analyses based on monoclonally inherited Y chromosome markers can overestimate gene flow when males are the dispersing sex (ALTHEIDE, T. K., pers. comm.). As such analyses based on autosomal markers that are randomly distributed throughout the taxa's nuclear genome and whose independent recombination events produce reticulate relationships at the population level, should reflect a more accurate genetic history of natural populations than uniparentally inherited markers (see BALL et al., 1990).

Interestingly, our findings agree with those of ZHI et al. (1996) in that they suggest that gene flow among Bornean demes is uninhibited. We, however, propose human interference, and not natural dispersal, as the principal source of gene flow. Our data, like that of WARREN et al. (2001), suggest that there are significant differences between the Northern Bornean and Sumatran Orangutans. Based on THORPE's (1982) criteria for determining levels of speciation based on genetic estimates derived from segregating loci, we propose that there are at least two subspecific groups of orangutans in Borneo: a Central Kalimantan-Sarawak clade and a Sabah-Northeast Kalimantan clade. In accordance with THORPE's (1982) criterion that conspecifics exhibit values of genetic identity (I) [where, $D = -\ln I$ (NEI, 1987)] greater than 0.85, our own estimates of I (range 0.54 – 0.85) based on SSR loci might qualify recommendations for the restructuring of the orangutan taxonomy.

Despite the vast geographic distribution, diversity of habitats and densities, a homogenous array of alleles across loci, albeit at very different frequencies, are maintained among most of the orangutan subpopulations. While the terrain that separates the Bornean demes provides no significant barriers to gene movement in the region, the level of gene glow is insufficient to offset completely the effects of genetic drift on the distribution of SSR alleles. Some of the gene flow, especially that between locales on the extreme ends of Borneo, and that between Borneo and Sumatra, which have been physically separated for over a million years (ZHI et al., 1996; KANTHASWAMY et al., in press), is probably human assisted. It is significant that the Central Kalimantan orangutans appear most genetically similar to Sumatran Orangutans because it probably reflects the effects of the Indonesian government's inter-island translocation programs. Although more contemporary individuals need to be studied to clarify the present population structure, the data presented here are suggestive of extensive genetic divergence, due to genetic drift, whose magnitude is nevertheless limited by natural or human assisted gene flow. If human intervention has irrevocably altered the natural distribution of orangutan genetic variation (i.e.

the subpopulations within Sumatra and Borneo are already genetically admixed), it may be too late to treat these demes as separate taxonomic units.

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