Molecular Ecology (2005) **14**, 441–456 doi: 10.1111/j.1365-294X.2004.02421.x

Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia

B. GOOSSENS,*†‡§ L. CHIKHI,§ M. F. JALIL,*‡ M. ANCRENAZ,† I. LACKMAN-ANCRENAZ,† M. MOHAMED,‡ P. ANDAU¶ and M. W. BRUFORD*

Biodiversity and Ecological Processes Group, Cardiff School of Biosciences, Cardiff University, Cardiff CF10 3TL, UK, †Kinabatangan Orang-utan Conservation Project, PO Box 3109, 90734 Sandakan, Sabah, Malaysia, ‡Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Locked Bag 2073, 88999 Kota Kinabalu, Sabah, Malaysia, §UMR 5174 Evolution et Diversité Biologique, Bâtiment 4R3b2, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cédex 4, France, ¶Sabah Wildlife Department, Muis Complex, 88100 Kota Kinabalu, Sabah, Malaysia

Abstract

We investigated the genetic structure within and among Bornean orang-utans (*Pongo pygmaeus*) in forest fragments of the Lower Kinabatangan flood plain in Sabah, Malaysia. DNA was extracted from hair and faecal samples for 200 wild individuals collected during boat surveys on the Kinabatangan River. Fourteen microsatellite loci were used to characterize patterns of genetic diversity. We found that genetic diversity was high in the set of samples (mean H_E = 0.74) and that genetic differentiation was significant between the samples (average F_{ST} = 0.04, $P < 0.001$) with F_{ST} values ranging from low (0.01) to moderately large (0.12) values. Pairwise F_{ST} values were significantly higher across the Kinabatangan River than between samples from the same river side, thereby confirming the role of the river as a natural barrier to gene flow. The correlation between genetic and geographical distance was tested by means of a series of Mantel tests based on different measures of geographical distance. We used a Bayesian method to estimate immigration rates. The results indicate that migration is unlikely across the river but cannot be completely ruled out because of the limited F_{ST} values. Assignment tests confirm the overall picture that gene flow is limited across the river. We found that migration between samples from the same side of the river had a high probability indicating that orang-utans used to move relatively freely between neighbouring areas. This strongly suggests that there is a need to maintain migration between isolated forest fragments. This could be done by restoring forest corridors alongside the river banks and between patches.

Keywords: genetic diversity, immigration, microsatellites, noninvasive sampling, *Pongo pygmaeus*, population fragmentation

Received 3 August 2004; revision received 19 October 2004; accepted 22 October 2004

Introduction

Current trends in great ape populations indicate a dramatic ongoing decline, which is predicted to result in the extinction of ape species in the wild for entire regions in the near future. Recent findings have particularly focused on African apes, and have implicated multiple factors, such as deforestation, hunting and disease (Walsh *et al*. 2003; Leendertz *et al*. 2004; Leroy *et al*. 2004). Less well publicised, but equally dramatic, has been the decline in Asia's only great ape, the orang-utan species of Sumatra and Borneo (*Pongo abelii* and *Pongo pygmaeus*). Current trends suggest that extinction is potentially imminent for the Sumatran species in the wild and although anthropogenic pressures are equally severe in parts of the orang-utan's range in Borneo, some potentially viable populations remain.

On both islands, orang-utans exist now mainly in fragmented and isolated populations, the sizes of which are

Correspondence: Benoît Goossens, Fax: + 33 5 61 55 73 27; E-mail: goossensbr@cardiff.ac.uk

only now being accurately estimated. Using wild reserve data, Rijksen & Meijaard (1999) estimated that the number of orang-utans may have dropped from *c*. 315 000 around 1900 to *c*. 27 000 in 1997 and have recently been estimated to be as low as 3500 in Sumatra (Wich *et al*. 2003). In Borneo, orang-utans appear to be widely distributed across Indonesia (Central, West, and East Kalimantan) and Malaysia (Sarawak and Sabah). Still, the situation appears critical as the population is estimated to have dropped from 23 000 in 1996 to 15 000 individuals in 1997 (a reduction of some 33% in one year, Rijksen & Meijaard 1999) because of drought and fires. Despite the uncertainties existing on these population size estimates (see Payne 1987, 1988), there is general agreement that populations have decreased at least 10-fold in the last 100 years (Delgado & van Schaik 2000).

There appears to be several causes for the dramatic decline of the Bornean orang-gutan in the last century, but it is known that processes threatening orang-utan populations include hunting, habitat loss, habitat degradation and forest fragmentation (Delgado & van Schaik 2000; Robertson & van Schaik 2001). In the Malaysian state of Sabah in the northern part of Borneo, human pressure has steadily increased. Indeed, in the last 20 years very large areas of forest have been logged and converted into oil palm plantations. In 2001, it was estimated that between a third and half of the original forest area had disappeared (McMorrow & Talip 2001).

Throughout their range, great apes, in common with many other species are increasingly affected by anthropogenic forest fragmentation and this problem is a major issue in Malaysia and Borneo (e.g. Laidlaw 2000; Kinnaird *et al*. 2003). Conservation planning for these increasingly isolated forest fragment communities presents a demanding set of challenges, ranging from determining viable population sizes (which may be in the tens of thousands for orang-utans, Harcourt 2002), assessing the potential for and importance of dispersal among populations (e.g. Travis & Dytham 1999) and estimating the relative importance of different ecological and life history parameters in predicting extinction risk (e.g. Brashares 2003). Large-bodied, slow-reproducing species, such as the orang-utan, have been shown in many studies to be more prone to extinction (Webb *et al*. 2002; Cardillo 2003), especially in closed or fragmented habitats (e.g. Laurance 1991; Davies *et al*. 2000). However, predicting population persistence is complex and, for example, those species which are able to effectively utilize modified habitats may remain stable or even increase in fragmented landscapes (Laurance 1991). Further, dispersal behaviours may modify according to habitat availability and persistence, potentially affecting, for example, predictions of extinction/recolonization in metapopulations (Travis & Dytham 1999; Reed 2004).

One trait which has rarely been examined in large-bodied species living in fragmented populations is genetic diversity. While the link between genetic diversity and population persistence has been demonstrated in smaller bodied vertebrates, invertebrates and plants (e.g. Saccheri *et al*. 1998; Madsen *et al*. 1999; Pryor *et al*. 2001) studies are much less common in large vertebrates, probably because of the fact that their slower vital rates are not expected to result in measurable reductions in genetic diversity over the timescale (in generations) of most anthropogenic habitat fragmentation (but see, for example, Miller & Waits 2003).

However, while genetic diversity may not obviously be affected in such species using standard gene diversity measures, such effects may be discernible in the genealogical data found in their allele distributions (e.g. O'Ryan *et al*. 1998; Goossens *et al*. submitted) and, regardless, genetic diversity in present day populations still needs to be managed judiciously in these species to guarantee their persistence in the future. For example, in the absence of direct behavioural observation, genetic methods can be used to infer dispersal and immigration events which can have profound consequences for population viability (e.g. Keller *et al*. 2001), allow the assignment of sexed individuals to their natal populations (e.g. Berry *et al*. 2004; Möller & Beheregaray 2004) and permit the development of a better understanding of how geographical features in different landscapes correlate with dispersal and genetic differentiation among local populations (e.g. Palsson 2004).

Orang-utans are large-bodied, semisolitary and slow-reproducing species, with extreme sexual dimorphism in body size and appearance. Orang-utans also show a pronounced bimaturism among sexually mature males and matings seem to be promiscuous, with both morphs (flanged and unflanged males) being reproductively successful in the populations (Rodman & Mitani 1987; Delgado & van Schaik 2000; Utami *et al*. 2002). Sexual maturity is variable and difficult to determine in the wild, particularly for males. In females it may vary between 7 and 15 years and is probably greater than 10 years in males (Leighton *et al*. 1995; Delgado & van Schaik 2000). Females care for dependent offspring for at least six years and the interbirth interval is about 8 years (Leighton *et al*. 1995). Slow growth and development contribute to a long lifespan, estimated to be about 45 years for both sexes in the wild (Leighton *et al*. 1995). Little is known about dispersal. Maturing females tend to remain near the natal area (philopatry), while males move away (Mitani 1989; Galdikas 1995; Singleton & van Schaik 2001). However, they seem to be very poor dispersers and they can be confined in isolated populations (van Schaik *et al*. 2001).

Within this context we studied the genetic diversity of an important remaining orang-utan 'population' in Sabah, found in the forests of the Lower Kinabatangan flood plain (*c*. 1100 individuals, Ancrenaz *et al*. 2004). In this area, conversion of forest into oil palm plantations has resulted in a highly fragmented forest structure (Fig. 1, Rijksen & Meijaard

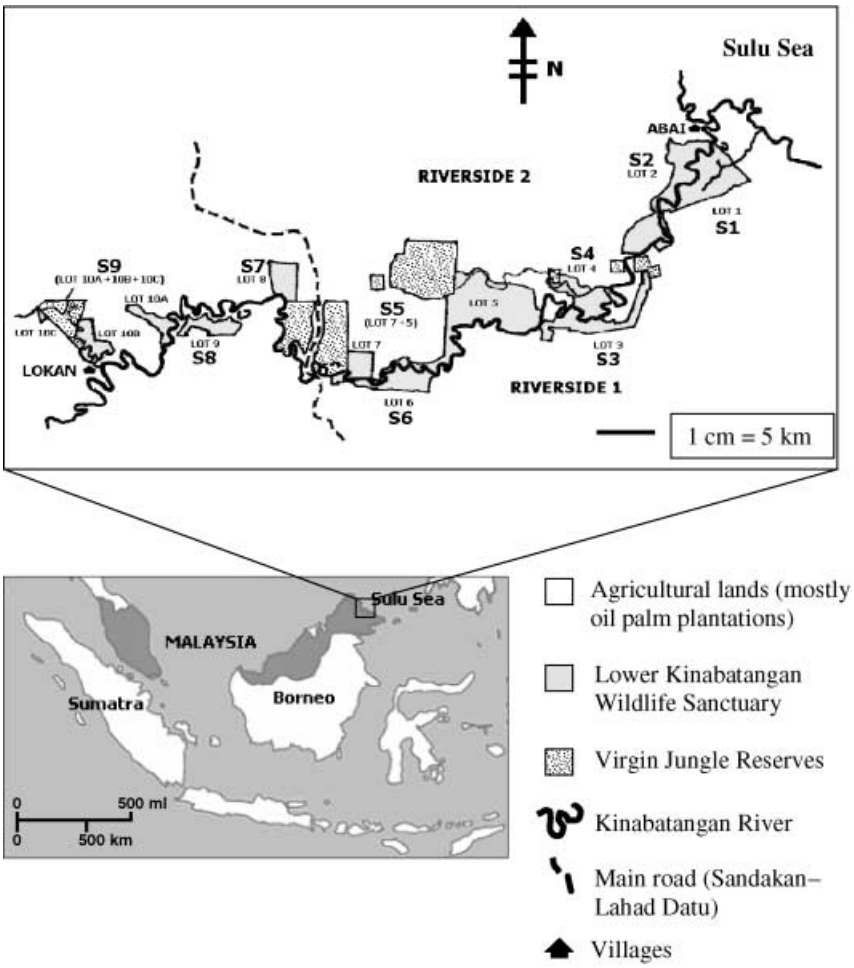

Fig. 1 Map of the Lower Kinabatangan Wildlife Sanctuary (LKWS) showing the location of the 10 lots of forests and the virgin jungle forest reserves alongside the Kinabatangan River. The inside map shows the location of the LKWS in Borneo Island.

1999; McMorrow & Talip 2001). In 2002, the state government of Sabah gazetted 27 000 ha of these forests as a wildlife sanctuary, with the ultimate aim of creating a corridor for wildlife along the Lower Kinabatangan flood plain, between the remaining virgin forest reserves. The impact of habitat fragmentation on the long-term survival of isolated orang-utan subpopulations is the main focus of current ecological and behavioural surveys in the region (Lackman-Ancrenaz *et al*. 2001). Population densities are unusually high for secondary forest, perhaps a result of recent habitat loss and consecutive concentration of individuals in the remaining forests (Ancrenaz *et al*. 2004).

While Bornean orang-utans have already been genetically studied (e.g. Zhi *et al*. 1996; Warren *et al*. 2000, 2001), the present study is the first to be carried out on wild animals (as opposed to individuals mostly sampled in zoos or in and around rehabilitation centres). Two other important specificities of the present work are: (i) the large number of individuals and loci typed (14 loci typed for 200 individuals), and (ii) the high proportion — *c*. 20% — that these individuals represent compared to the estimated number of individuals present in the sampled region (Ancrenaz *et al*. 2004). Specifically, we examine genetic structure within and among the remaining sampled forest fragments and determine the effect of natural barriers such as the Kinabatangan River, on isolation. We estimated diversity within and migration rates among forest patches on the same and different sides of the river. Our analysis includes an attempt to assess the genetic effects of both past and ongoing dispersal. The applicability of these data to be incorporated in conservation assessment in a management plan for *P. pygmaeus* in the region is discussed.

Materials and methods

The Lower Kinabatangan flood plain and the Lower Kinabatangan Wildlife Sanctuary

The Lower Kinabatangan flood plain (5 20′–5 45′ N, 117 40′–118 30′ E) is located in eastern Sabah, Malaysia. The flood plain is a patchwork of different habitat types: riverine forest, seasonally flooded forest, swamp forest,

dry dipterocarp forest, nipa palms, and mangrove (Azmi 1998). However, since the mid 1950s, the whole Lower Kinabatangan region has been subjected to large-scale commercial timber exploitation and agriculture. During the past 20 years, postlogging land conversion to oil palm plantations has been extensive (McMorrow & Talip 2001).

On 16 January 2002, the proposed Lower Kinabatangan Wildlife Sanctuary (LKWS) was gazetted and now comprises 10 sectors or lots (lots 1–10, with lot 10 divided into 10A–C) chosen to increase connectivity between remaining forest reserves (Fig. 1). The aim of this sanctuary is to transform the 27 000 ha of flood plain into a forest corridor connecting the coastal mangrove swamps with dry land forests upriver.

Sampling

Shed hair in nests and faeces were collected from wild orang-utans during boat surveys carried out alongside the Kinabatangan River (between Abai village and Lokan village, corresponding to a 280 km river tract, see Fig. 1) between January and May 2001. When a fresh nest (between one and five days old – see Goossens *et al*. 2004) was spotted, shed hairs were collected. Shed hairs were also collected during line transects made to estimate nest densities (Ancrenaz *et al*. 2004). Faecal samples found below fresh nests were collected as well. When an orang-utan was directly encountered it was followed until defecation and the faecal sample was collected.

Hair samples were stored in plastic bags, whereas faecal samples were stored in 50 mL BD Falcon™ tubes with 90% ethanol. Precautions were taken to avoid human contamination during the sampling by using sterile gloves and implements (sterilized forceps). GPS coordinates were taken for each sample.

Shed hairs from 176 different nests, and faecal samples from 71 orang-utans were collected and could be assigned to nine sampling regions S1–S9 (Fig. 1), which corresponds mostly to the lots described above, except that samples in lots 5 and 7 were grouped into S5. In the 32 cases where faecal samples were collected below fresh nests, they were used instead of shed hairs collected in the nest. Thus, of a total of 279 samples collected, 247 samples were selected for genetic analyses.

DNA extraction

For shed hairs (144 samples), DNA was extracted using a polymerase chain reaction (PCR) buffer method (Vigilant 1999). Faecal extractions (103 samples) were carried out in a Class I microbiological safety hood, using the QIAamp DNA Stool Mini Kit (QIAGEN) and following a protocol for orang-utans detailed in Goossens *et al*. (2000a) and Utami *et al*. (2002).

Table 1 Characteristics of 14 human-derived microsatellite loci used in *Pongo pygmaeus*

Locus ID	T_a (C), time (s)	Size (bp)
D5S1457	49, 45	111–139
D5S1470	51, 30	208–236
D1S550	60, 30	128–166
D2S1326	60, 30	200–224
D3S2459	60, 45	200–216
D4S1627	55, 30	188–208
D4S2408	64, 45	274–306
D5S1505	64, 30	211–243
D6S501	60, 30	153–181
D13S321	60, 45	200–216
D13S765	60, 45	185–205
D12S375	60, 30	172–188
D2S141	64, 45	138–150
D16S420	58, 30	178–194

T_a = optimal PCR annealing temperature.

DNA amplification and microsatellite genotyping

Fourteen human-derived microsatellite loci were used: 2 dinucleotide loci D2S141 and D16S420; and 12 tetranucleotide loci D5S1457, D5S1470, D1S550, D2S1326, D3S2459, D4S1627, D4S2408, D5S1505, D6S501, D13S321, D13S765 and D12S375 (Table 1) (see also Coote & Bruford 1996; Goossens *et al*. 2000b, 2002; Zhang *et al*. 2001; Utami *et al*. 2002). All forward primers were fluorescently labelled. All PCR reactions were carried out in 12.5 L total containing 2.5 L DNA extract. A multiple-tube procedure was conducted for each faecal extract according to Taberlet *et al*. (1996). For each extract, three amplifications were performed using the D5S1457 locus (Goossens *et al*. 2000a). After that, the most successful extract (three positive PCRs) for each sample was amplified seven times for each locus to avoid typing errors (see Taberlet *et al*. 1999 for a review). Amplifications were carried out in 12.5 L [10 mM Tris-HCl (pH 9.0), 200 mM $(NH_4)_2SO_4$, 50 M each dNTP, 1.5 mM $MgCl_2$, 5 ng of BSA, 0.1 U Ampli*Taq* Gold DNA polymerase (Perkin Elmer), 0.5 M reverse primer, 0.5 M fluorescent (TET, FAM or HEX) forward primer, 2.5 L of DNA extract]. PCR amplification of 50 cycles was carried out for each locus separately (initial denaturation 94 C for 10 min, 94 C for 15 s, 45 C to 52 C for 15–30 s, 72 C for 30–60 s). The annealing temperature was optimized for each locus (Table 1). All PCR products were separated on an acrylamide gel using an ABI PRISM 377 DNA sequencer. Gels were analysed using GENESCAN ANALYSIS 2.0 and GENOTYPER 1.1 software.

Genetic diversity and population structure

Genetic diversity was measured as the mean number of alleles per locus (MNA), observed (H_O), and expected (H_E)

heterozygosities (Nei 1978). Linkage disequilibrium (LD) was estimated across all pairs of loci using the correlation coefficient of Weir (1979). A permutation approach was used to determine which LD values were significant. Wright's *F* statistics were estimated according to Weir & Cockerham (1984) and their departure from the null hypothesis (no genetic differentiation for F_{ST}, and Hardy–Weinberg equilibrium for F_{IS} and F_{IT}) was tested using permutations. Analyses were performed using the GENETIX software (Belkhir *et al*. 1996/1997, available at http://www.University-montp2.fr/~genetix/genetix/genetix.htm).

Differentiation between river sides

As the Kinabatangan River represents a natural barrier to the movement of orang-utans, we divided the samples into two sets called River Side 1 — RS1 = (S1, S3, S6, S8) — and River Side 2 — RS2 = (S2, S4, S5, S7, and S9). We then looked at the distribution of pairwise F_{ST} values between samples belonging (i) to the same side of the river (RS1 vs. RS1 and RS2 vs. RS2) and (ii) to different sides (RS1 vs. RS2). We then compared them to the set of all pairwise F_{ST} values (i.e. regardless of the river side). For simplicity of notation we shall refer to these sets as $F_{ST(RS1)}$, $F_{ST(RS2)}$, $F_{ST(RS1-2)}$, and $F_{ST(TOT)}$, respectively. We were interested in two different statistical tests. In the first, we compared the $F_{ST(RS1)}$, $F_{ST(RS2)}$ and $F_{ST(RS1-2)}$ distributions to the $F_{ST(TOT)}$ distribution. This allowed us to determine whether each subset was significantly different from a random subsample of all the F_{ST} values. This was done by repeatedly permuting the set $F_{ST(TOT)}$ values to create three sets of F_{ST} values containing the same number of F_{ST} values as $F_{ST(RS1)}$, $F_{ST(RS2)}$ and $F_{ST(RS1-2)}$, respectively. At each permutation and for each set we calculated the mean F_{ST} value. The observed (real) average F_{ST} of each subset was then compared to the distributions obtained. In the second set of tests, we compared the $F_{ST(RS1)}$ and $F_{ST(RS2)}$ distributions to the distribution of $F_{ST(RS1-2)}$ values. The randomization was done by sampling from the distribution of $F_{ST(RS1-2)}$ values one subset of F_{ST} values with the same size as the $F_{ST(RS1)}$ (or $F_{ST(RS2)}$) set. The distribution of means was then compared to the real mean. This allowed us to test whether F_{ST} values within each river side were significantly lower than those observed between river sides.

In order to further assess the effect of the river in the patterns of genetic differentiation and to test the correlation between genetic and geographical distance, a series of two-way Mantel tests was carried out (Mantel 1967). In each of these tests the matrix of pairwise F_{ST} values was used against four different matrices of geographical distances. The four geographical distances were built in order to account for the potential role of the Kinabatangan as a geographical barrier. In the first case, the river was ignored and a simple Euclidian distance was computed among all samples. In the three other cases three different assumptions were made regarding the point at which orang-utans were possibly able to cross the river, whereas distances between samples from the same side were computed by following the river. The three hypothetical crossing points were assumed to be (i) at the level of S8 and S9 (the most upstream samples used) where the river is approximately 200 m wide, (ii) approximately 150 km upstream of S8 and S9, which is probably the closest location where the river is narrow enough to allow orang-utans to use fallen trees to cross the river, and (iii) at the Kinabatangan source, approximately 260 km upstream of S8 and S9. All the permutation tests above, including the Mantel test, were performed using the *R* statistical package.

Immigration between river sides

Wilson & Rannala (2003) recently developed a Bayesian method to estimate rates of recent immigration in a set of linked populations using multilocus data. The method is based on a simple model where individuals are exchanged between populations over generations. The probability of observing a particular genotype in a given population can be expressed as a function of the model's demographic parameters (this probability is the likelihood). These parameters include the allele frequencies, the immigration rates (m_{ij}, the proportion of individuals in population j that originate from population i), the inbreeding level in each population (F_i being the inbreeding in population i), and the time at which the immigration event took place (the method currently accounts for immigration events taking place either at the sampling generation t_1, or one generation before, t_2). Based on this likelihood function, Wilson & Rannala (2003) use a Markov chain Monte Carlo (MCMC) approach to explore the parameter space and obtain samples from the posterior distributions of the parameters of interest. One interesting property of this method is that, contrary to most methods currently available, it does not require samples to be at Hardy–Weinberg equilibrium (HWE). Also, an advantage over assignment methods is that migration events are accounted for in the calculation of allele frequencies, and hence in the likelihood. This is typically ignored by assignment methods. Finally, it is important to note that the method allows to estimate immigration rather than migration rates.

The method is implemented in the software BAYESASS (http://www.rannala.org/labpages/software.html). The software allows the user to change parameters affecting the proposal distributions, namely *deltap*, *deltam*, and *deltaF*, which define the manner in which the parameter space is explored during the MCMC (details on the proposal distributions can be found in Wilson & Rannala 2003). Using different values as we did can be crucial as some choices could produce sticky Markov chains that take a long time to

converge (e.g. Gilks *et al*. 1996). Different summaries of MCMC runs and some parameter distributions can be saved and examined. One reason for saving only summaries rather than the chains is that the number of parameters of potential interest can grow very quickly with the number of populations. However, we found that not having access to the chains could be problematic (see below). In particular, it can be critical to check that equilibrium has been reached before using summaries (e.g. Chikhi *et al*. 2001). The code was therefore modified and recompiled to produce an output with the F_i and the m_{ii}, thus producing outputs with $2n$ columns for data from n populations (i.e. for each step of the MCMC a line with the n following numbers is produced: $F_1 \ldots F_n\ m_{11}\ m_{22} \ldots m_{nn}$). The modifications of the code and the corresponding executable were provided to G. Wilson and can be obtained from him. The results presented here for the comparison of the two river sides were obtained from five independent runs with different starting values for the random number generator and different values of the proposal distributions (the parameters *deltap*, *deltam* and *deltaF* varied between 0.05 and 0.35). Wilson & Rannala (2003) ran their data for 3×10^6 iterations discarding the first 10^6 as a burn-in. We first used these conditions but decided to increase the total number of iterations to check convergence on very long runs. The number of iterations were 10, 15 (two runs) and 20×10^6. In order to test whether our modifications of the code did not make any change, the data were run for 20×10^6 iterations and the results compared to the original ones from the 5 runs. They were not distinguishable from them.

Assignment tests

Assignment tests were also performed on the data to determine whether it was possible to assign individuals to their population or river side of origin. Different approaches have been proposed to estimate assignment probabilities (e.g. Paetkau *et al*. 1995; Cornuet *et al*. 1999). We applied the method of Rannala & Mountain (1997) because it has been shown to provide the best assignment results by simulations (Cornuet *et al*. 1999). Note that most methods usually provide similar results unless sample sizes (in terms of individuals and number of loci typed) are small. Because we are interested in the role of the Kinabatangan River as a barrier to orang-utans movement, the exact method chosen was not crucial. This point is discussed below (see Cegelski *et al*. 2003; Berry *et al*. 2004; and references therein for in-depth analysis and comparison of assignment methods). Rannala and Mountain's method is implemented in the GENECLASS software. Simulations were used to rank *'populations'* (i.e. samples S1 to S9) and determine the most probable sources for all 200 individuals. For each individual, we then checked whether the most probable, second most probable, … sample was from the same river side as the individual analysed. Using only the most probable source is not necessarily a good choice given that the second most probable could be from the other side. We thus decided to apply a 'majority rule' algorithm and check the most probable river side among the k most probable samples. The value of k could in principle be any value between 1 and 9. However, the value $k = 1$ corresponds to choosing the most probable sample. Taking $k = 9$ will lead us to take all populations which would not make sense either. Given that there are four samples from RS1 and five from RS2, even if the assignment was perfect, the most probable sample from the opposite river side would necessarily appear on the 5th and 6th rank, respectively. This also means that the majority rule must account for the higher probability of having an individual assigned to RS2 by chance. For individual from RS1 the majority rule applies if more than 4/9th of k comes from RS1, whereas for individuals from RS2, the rule applies if more than 5/9th of k individuals are assigned to RS2. Because k cannot be too small or too large for the reasons given above, we decided to apply the majority rule to the first five and six samples in the assignment ranking.

Results

Genetic typing

We were able to reliably amplify DNA from 201 out of 247 samples. Two individuals had the same genotype at all 14 loci and corresponded to samples taken from two fresh nests *c.* 100 m apart. With the exclusion of this case, no other pair of samples had the same genotypes at the 14 loci. We found one pair of individuals identical at 13 loci and another pair identical for 12 loci. Two pairs were identical at 11 loci and three pairs had 10 loci in common. While we cannot exclude the possibility that the two genotypes above were from different individuals, they were considered to be from the same individual, leaving a total of 200 different individuals, corresponding to a total of 200*14 = 2800 genotypes. Of these only seven genotypes (0.25%) were not reliable using the multiple-tubes approach and were therefore coded as missing genotypes.

Genetic diversity, departure from Hardy–Weinberg equilibrium (HWE) and LD

All the 14 loci used in the study were polymorphic, with between five and nine alleles per locus across all samples (Table 2). The mean number of alleles (MNA) per locus ranged between 4.1 (S3) and 4.9 (S4, S5 and S9); the lowest was 3.3 (S7, which only has a sample size of five). Average H_E values were high (0.66–0.75, Table 2). Average H_O values were slightly higher (0.67–0.77), generating slightly

Table 2 Average number of alleles across samples (N_a), observed (H_O) and expected (H_E) heterozygosities and departures from Hardy–Weinberg proportions (F_{IS}) for all samples and for all loci

Locus	Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	
	n	27	26	22	20	27	33	5	24	16	N_a
D5S1457	H_E	0.766	0.773	0.637	0.663	0.718	0.734	0.778	0.699	0.804	7
	H_O	0.852	0.654	0.727	0.600	0.593	0.818	1.000	0.667	0.750	
	F_{IS}	0.115	0.157	–0.145	0.097	0.178	–0.116	–0.333	0.047	0.070	
		NS	NS	NS	NS	NS	NS	***	NS	NS	
D5S1470	H_E	0.750	0.724	0.802	0.685	0.744	0.733	0.644	0.756	0.752	8
	H_O	0.963	0.769	0.727	0.800	0.667	0.758	0.600	0.750	0.562	
	F_{IS}	0.290	–0.064	0.096	–0.174	0.105	–0.034	0.077	0.008	0.258	
		***	NS	NS	NS	NS	NS	NS	NS	NS	
D1S550	H_E	0.757	0.744	0.646	0.745	0.789	0.772	0.689	0.551	0.754	7
	H_O	0.778	0.692	0.773	0.750	0.556	0.697	0.600	0.458	0.750	
	F_{IS}	0.027	0.070	–0.202	–0.007	0.300	0.098	0.143	0.172	0.006	
		NS	NS	NS	NS	**	NS	NS	NS	NS	
D2S1326	H_E	0.746	0.762	0.780	0.700	0.723	0.792	0.711	0.840	0.720	7
	H_O	0.815	0.731	0.864	0.526	0.556	0.849	0.800	0.708	0.625	
	F_{IS}	0.094	0.041	–0.110	0.253	0.235	–0.072	–0.143	0.159	0.135	
		NS	NS	NS	NS	*	NS	NS	NS	NS	
D3S2459	H_E	0.640	0.782	0.624	0.696	0.735	0.731	0.733	0.726	0.740	5
	H_O	0.778	0.654	0.682	0.600	0.630	0.697	0.800	0.667	0.750	
	F_{IS}	0.220	0.167	–0.096	0.141	0.146	0.047	–0.103	0.083	–0.014	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
D4S1627	H_E	0.665	0.647	0.680	0.768	0.693	0.717	0.689	0.719	0.796	6
	H_O	0.815	0.769	0.727	0.750	0.704	0.758	0.800	0.875	0.812	
	F_{IS}	0.231	–0.193	–0.072	0.024	–0.016	–0.058	–0.185	–0.223	–0.021	
		**	NS	NS	NS	NS	NS	NS	*	NS	
D4S2408	H_E	0.658	0.624	0.537	0.735	0.639	0.693	0.533	0.662	0.704	5
	H_O	0.630	0.577	0.636	0.700	0.741	0.667	0.800	0.708	0.625	
	F_{IS}	0.043	0.076	–0.190	0.048	–0.162	0.039	–0.600	–0.071	0.115	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
D5S1505	H_E	0.736	0.768	0.724	0.817	0.778	0.788	0.600	0.840	0.827	9
	H_O	0.926	0.769	0.773	0.800	0.808	0.758	0.800	0.792	0.625	
	F_{IS}	0.265	–0.001	–0.069	0.021	–0.039	0.039	–0.391	0.059	0.250	
		**	NS	NS	NS	NS	NS	***	NS	**	
D6S501	H_E	0.547	0.622	0.506	0.760	0.698	0.626	0.622	0.622	0.706	8
	H_O	0.556	0.654	0.545	0.850	0.704	0.636	0.600	0.708	0.875	
	F_{IS}	0.016	–0.052	–0.079	–0.122	–0.008	–0.017	0.040	–0.143	–0.250	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
D13S321	H_E	0.800	0.737	0.627	0.796	0.792	0.792	0.600	0.798	0.772	5
	H_O	0.808	0.731	0.636	0.800	0.741	0.758	0.800	0.792	0.875	
	F_{IS}	0.010	0.008	–0.016	–0.005	0.066	0.044	–0.391	0.008	–0.138	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
D13S765	H_E	0.752	0.581	0.688	0.717	0.705	0.707	0.467	0.728	0.514	6
	H_O	0.731	0.577	0.773	0.800	0.667	0.697	0.600	0.750	0.438	
	F_{IS}	0.029	0.007	–0.126	–0.120	0.055	0.015	–0.333	–0.031	0.153	
		NS	NS	NS	NS	NS	NS	***	NS	NS	
D12S375	H_E	0.639	0.606	0.575	0.679	0.680	0.671	0.733	0.662	0.724	5
	H_O	0.741	0.731	0.455	0.850	0.704	0.697	0.800	0.833	0.812	
	F_{IS}	0.162	–0.212	0.213	–0.259	–0.036	–0.039	–0.103	–0.265	–0.127	
		NS	NS	NS	*	NS	NS	NS	*	NS	
D2S141	H_E	0.677	0.594	0.809	0.768	0.722	0.760	0.778	0.802	0.782	6
	H_O	0.704	0.577	0.818	0.800	0.778	0.758	0.800	0.913	0.812	
	F_{IS}	0.040	0.028	–0.012	–0.043	–0.079	0.004	–0.032	–0.142	–0.040	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
D16S420	H_E	0.686	0.664	0.638	0.759	0.804	0.744	0.778	0.784	0.855	8
	H_O	0.680	0.577	0.727	0.800	0.852	0.697	1.000	0.833	0.938	
	F_{IS}	0.009	0.133	–0.143	–0.056	–0.060	0.064	–0.333	–0.065	–0.100	
		NS	NS	NS	NS	NS	NS	NS	NS	NS	
Total											
	H_E	0.701	0.688	0.662	0.735	0.730	0.733	0.668	0.728	0.746	
	H_O	0.770	0.676	0.705	0.745	0.693	0.732	0.771	0.747	0.732	
		0.099	0.017	–0.065	–0.014	0.052	0.002	–0.177	–0.027	0.020	
	F_{IS}	***	NS	NS	NS	NS	NS	*	NS	NS	
	MNA	4.500	4.714	4.143	4.857	4.857	4.714	3.286	4.786	4.929	

NS = nonsignificant, * = $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. *n* = sample size.

Table 3 Pairwise F_{ST} values

	S1	S2	S3	S4	S5	S6	S7	S8	S9
S1 (n = 27)	0.000	0.051	0.029	0.056	0.040	0.015	0.075	0.022	0.045
S2 (n = 26)	***	0.000	0.074	0.038	0.014	0.037	0.022	0.061	0.042
S3 (n = 22)	***	***	0.000	0.092	0.067	0.028	0.120	0.027	0.079
S4 (n = 20)	***	***	***	0.000	0.013	0.046	0.029	0.065	0.019
S5 (n = 27)	***	**	***	*	0.000	0.028	0.014	0.054	0.015
S6 (n = 33)	***	***	***	***	***	0.000	0.049	0.018	0.033
S7 (n = 5)	***	NS	***	*	NS	**	0.000	0.079	0.020
S8 (n = 24)	***	***	***	***	***	***	***	0.000	0.038
S9 (n = 16)	***	***	***	**	*	***	NS	***	0.000

NS = non significant, * = $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n = sample size.

negative F_{IS} values (average $F_{IS} = -0.019$, NS) (Table 2). When all samples were considered, a significant departure from Hardy–Weinberg was observed with an average F_{IT} of 0.024 ($P < 0.01$). However, as the non significant F_{IS} values show, this appears to be mostly caused by differentiation between samples ($F_{ST} = 0.040$, $P < 0.001$) and is therefore probably the result of a Wahlund effect.

We found 95 significant LD values (at $\alpha = 0.05$) across all sampled regions (Appendix 1). S7 exhibited only two significant LD values, most probably because of its small sample size (n = 5). Most samples appear to have between six and 10 significant LD values, but S1 and S3 have 18 and 22 significant LD values, respectively. Despite this difference, we could not see any clear pattern across samples. For instance, no pair of loci was exhibiting a significant LD in more than three samples, the average being 1.07 population per locus pair with some variation across regions. For instance S1 and S3 only share three pairs of loci in LD. These results indicate that LD is most likely a result of the demographic history of the populations including events such as admixture or drift, rather than linkage.

Genetic differentiation between samples and between river sides

Overall, we found a limited but significant level of genetic differentiation among the samples (average $F_{ST} = 0.04$, $P < 0.001$). Pairwise F_{ST} values range between 0.01 and 0.12 and most are significant (Table 3, Fig. 2). As described in the Materials and methods section, it is possible to divide the samples into two sets, RS1 = (S1, S3, S6, S8) and RS2 = (S2, S4, S5, S7, S9), to test whether the Kinabatangan River represents a natural barrier to the movement of orang-utans. The distribution of pairwise F_{ST} values between samples belonging (i) to the same side of the river ($F_{ST(RS1)}$ and $F_{ST(RS2)}$) and (ii) to different sides ($F_{ST(RS1\text{-}2)}$) can be compared to the set of all pairwise F_{ST} values. Histograms of these values are represented in Fig. 2. The figure shows that, on average, F_{ST} values between samples from the same side of the river (second and third panel from Fig. 2, average $F_{ST} = 0.023$ and 0.026, respectively) are lower than F_{ST} values between samples from different sides (lower panel of Fig. 2, average $F_{ST} = 0.058$). The permutation tests (all tests were significant at 0.1%, with the exception of the $F_{ST(RS1)}$ vs. $F_{ST(TOT)}$, which was significant at 5%) we performed allow us to demonstrate that (i) the three lower panels are not random sets of F_{ST} values, (ii) the F_{ST} values within each river side are significantly lower than the average F_{ST} across all samples, (iii) the F_{ST} values between river sides are significantly larger than the average F_{ST} across all samples, and (iv) the F_{ST} values within each river side are significantly lower than those observed between river sides.

Results of the Mantel tests performed with different measures of geographical distances (see Materials and methods) indicate that there is significant correlation between geographical and genetic distance when the river is considered to be a barrier ($r = 0.54, 0.72, 0.73$, $P < 0.01$, for the three distances used), but there is no correlation when the simple Euclidian geographical distance is used for all samples regardless of the river side ($r = -0.07$, NS). The correlation greatly increases between the case where we assume that orang-utans could cross at the level of S8 and S9 (where the river is still 200 m wide $r = 0.54$) and 150 km upstream (and the river starts to be reasonably narrow, $r = 0.72$). The correlation does not, however, increase with greater distance (i.e. when we assume that orang-utans could only cross the river at its source, $r = 0.73$).

Immigration rates

When samples were analysed using one river side vs. the other side, we found that the method of Wilson & Rannala (2003) produced highly consistent outputs across the runs, with clear indications that immigration rates are extremely low. For RS1, the posterior mean was 0.988, with the most probable value at 0.998 (Fig. 3). For RS2, the posterior mean was 0.971, with the most probable value at 0.982 (Fig. 3).

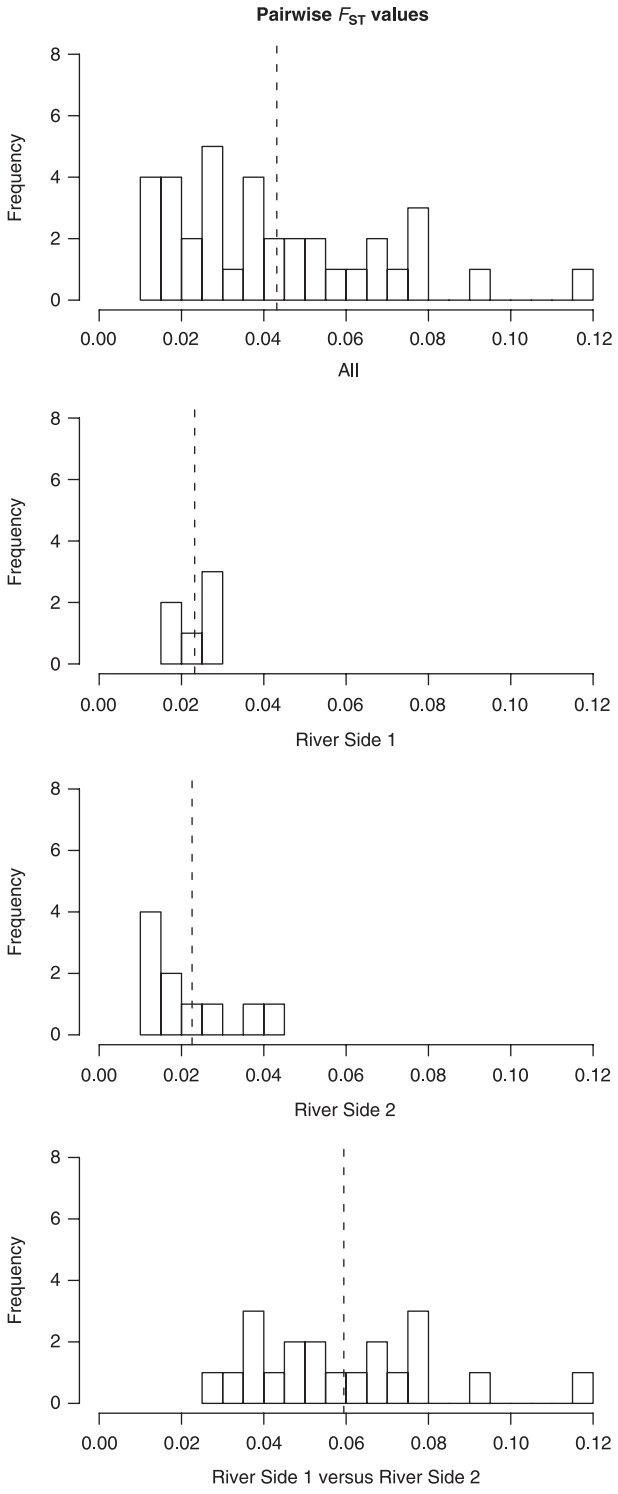

Fig. 2 Pairwise F_{ST} values. The top panel represents pairwise F_{ST} values between all populations. The second and third panels represent pairwise F_{ST} values either between samples from RS1 or from RS2, respectively (see text). The last panel corresponds to pairwise F_{ST} between samples from opposite river sides.

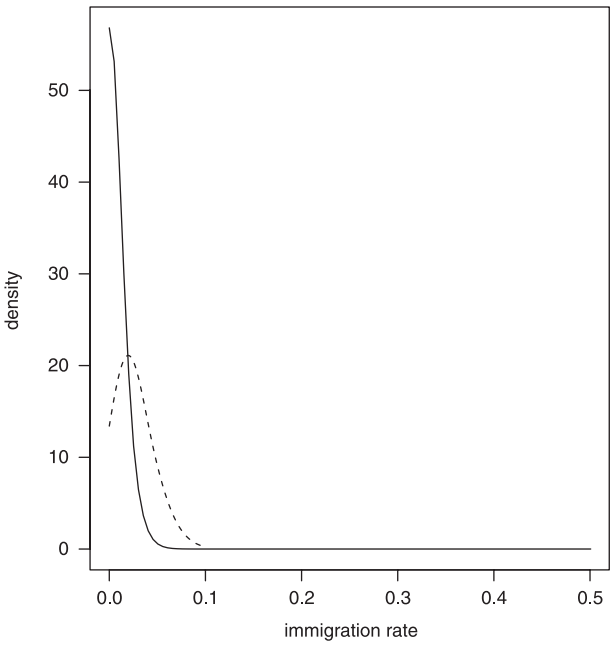

Fig. 3 Posterior distributions for the immigration rates for RS1 and RS2.The solid and dashed curves correspond to 1-m11 and 1-m22, and represent the immigration rate for RS1 and RS2, respectively.

Therefore, while it is impossible to completely reject the existence of movement across the river, there is very strong support for very low levels of migration despite the limited genetic differentiation. In fact, the method allows the determination for each individual (i.e. genotype) a posterior probability of being an immigrant. In such cases, it is also possible to estimate whether the multilocus genotype is an immigrant or the descendant of an immigrant in the preceding generation. These results show that for most individuals (156 out of 200), the probability of being a local rather than an immigrant is greater than 95%. For 41 of the remaining 44 individuals, this probability was not as high but remained larger than that of being an immigrant. For three individuals, all of which from RS2 (S4, S7 and S9), the probability of being an immigrant was larger than that of being local, even though the latter probability was still non-negligible (20, 40 and 44%, respectively). It is difficult to determine whether these individuals could indeed be immigrants or locals because of the limited F_{ST} values observed. In other words, most individuals are more likely to come from the river side they were sampled in, and for three individuals, the odds are that they could come from both sides.

When the method was applied using the nine samples (S1–S9) independently (i.e. allowing the estimation of immigration rates both within and between river sides), we found that the method produced inconsistent results across runs for some parameters, such as the F_i. In order to

determine whether these inconsistencies were a result of the lack of convergence of some parameter for some values of *deltap*, *deltam* or *deltaF* (i.e. inefficient proposal distributions), we modified the outputs (see Materials and methods). This allowed us to determine that migration rates were probably too high between samples from the same side to allow the method to work (this was confirmed by discussions with G Wilson). The method was therefore not used further at this scale. We note however, that the method is expected to become inefficient when immigration rates are greater than 66% (Wilson & Rannala 2003). Our results could thus indicate that individuals sampled in the different lots of each river side have relatively high probability of being immigrants from other neighbouring lots. This probability cannot be safely estimated because of the lack of convergence but is likely to be high.

The assignment analysis confirms these results but it indicates as well that for 11 individuals (five in RS1 and six in RS2), the most probable sample of origin is from the other river side. Applying the majority rule defined above, we find that among the five most probable samples, the river side of origin is more often represented in 168 individuals out 200. Applying the same rule to the six most likely sources increases this number to 181. In other words, there are between *c.* 20 and *c.* 35 individuals which are most probably assigned to the opposite river side.

Discussion

Genetic diversity, individual identification and population sizes

The results presented here show that the orang-utans sampled in the Lower Kinabatangan flood plain exhibit a high level of genetic variability despite the fragmentation of their environment. The diversity exhibited by the 14 human-derived microsatellite loci was high enough to permit an individual genetic identification of all 200 orang-utans typed in the study, which could prove particularly valuable for future studies of paternity assessment and relatedness.

Given the current census size estimates of approximately 1100 individuals (Ancrenaz *et al*. 2004), the genetic diversity observed in the Lower Kinabatangan orang-utans is surprisingly high, suggesting that orang-utans are not at mutation–drift equilibrium. This is supported by the relative lack of rare alleles, typically observed in populations that have been subject to a demographic bottleneck (e.g. Nei *et al*. 1975). Indeed, such populations are expected to first lose their rare alleles. As H_E is little affected by rare alleles (the square of their frequency is negligible), high H_E values can be observed long after the bottleneck has taken place (Nei *et al*. 1975; Chikhi & Bruford in press). These results are also in agreement with the very large numbers thought to have existed in the last centuries and millennia across Borneo (Rijksen & Meijaard 1999; Delgado & van Schaik 2000), but do not allow us to determine whether the decrease in orang-utan numbers is recent or ancient.

One possibility is that the signal we detect corresponds to the slow decrease of orang-utans since the Pleistocene because of a combination of climate change and prehistoric hunting (Delgado & van Schaik 2000). It is possible, as a rough approximation, to estimate the long-term effective size, N_e, compatible with the observed level of genetic diversity as measured by H_E. Under the stepwise mutation model and with a mutation rate between 10^{-3} and 10^{-4}, we find that N_e would have to be between *c.* 1500 and 17 000 (Ohta & Kimura 1973). While such N_e estimates should not be taken at their face value, they are higher than the census size estimates and therefore confirm that the variability present in the Lower Kinabatangan is 'surprisingly high'. This result is further confirmed by the fact that in using human-derived microsatellites, there is likely to be an ascertainment bias towards underestimating genetic diversity (Ellegren *et al*. 1997), and hence N_e, in orang-utans.

Another possibility is that the pattern of high diversity with few rare alleles and a small census size corresponds to much more recent changes, namely the anthropogenic destruction and fragmentation of the habitat that has taken place in the last century and in particular during the last decades. In the latter case, the high level of genetic diversity currently observed could be explained by three factors: (i) the presence of very large numbers throughout the Kinabatangan area over long periods of time, as noted above, (ii) the very recent habitat loss and degradation, which may have led to the concentration of the surviving individuals in the remaining forest patches along the river, and (iii) the long generation time and lifespan of the species which allowed populations to retain diversity for long periods after habitat loss. One consequence of this would be that high genetic diversity is transient and may only be present for a short time, as it would be 'concentrated' in adults which may soon be unable to reproduce.

A detailed exploration of past orang-utan demography has been carried out by Goossens *et al*. (submitted) and thus will not be developed here. We only note that when formal statistical tests are performed a strong and highly significant signal for a past demographic bottleneck is demonstrated confirming that the high level of genetic diversity observed in the Lower Kinabatangan is the remnant of an ancient large population.

Population structure, isolation by distance, and immigration rates

The analysis of the population structure showed a moderate (but significant) level of genetic differentiation between samples that are not geographically distant (average F_{ST} ~0.04). When only samples from the same side of the river

were analysed the average F_{ST} was significantly lower (F_{ST} ~0.025) than when samples from across the river were analysed (F_{ST}~0.06). These differences indicate that the Kinabatangan River represents a significant barrier to gene flow. The role of the Kinabatangan as a barrier is confirmed by our analysis of the correlation between pairwise F_{ST} values and the four geographical distances. When we assumed that orang-utans were able to cross the river in far upstream regions where the river's width becomes much smaller, we found that there was a significant correlation between geographical and genetic distance. This correlation disappeared when the river was artificially ignored. We also found that the correlation increased when we assumed that crossing the river was equivalent to travelling approximately 300 km. We thus recalculated the correlation between the two matrices by incrementing the distance from S8 and S9 to the crossing point by multiples of 10 km (corresponding to an increase of 20 km by going upstream for 10 km and back). We find that the correlation increases rapidly for the first 100 km (corresponding to an increase in distance of 200 km) from $r = 0.52–0.69$ but not after *c.* 150 km (i.e. 300 km in total).

Finally, the analysis of immigration rates allowed us to determine that rates of recent immigration were most probably close to zero across the river. We could not exclude the possibility that some individuals could have crossed the river and even found that assignment tests were sometimes favouring the opposite river side as the most probable area of origin. Practical knowledge of the sampled area suggests that it is extremely unlikely if not impossible for orang-utans to cross the Kinabatangan. The only bridge that could be used corresponds to the very frequented Sandakan-Lahad Datu road and is thus difficult to cross. Moreover, it would require the orang-utans to cross a village on one end. This suggests that the results obtained using the assignment method are either a result of the fact that intermediate genotypes can be 'generated' by both river sides (i.e. their likelihood is non-negligible using both riversides frequencies) or that they may come from other nonsampled regions. Another possibility is that they reflect the uncertainty resulting from the limited F_{ST} values between samples and river sides. In the immigration analysis this possibility is the most probable because of the decrease in average F_{ST} values obtained by pooling all samples from either river sides (the F_{ST} decreases from 0.058 to 0.036). Wilson & Rannala (2003) applied their method to two data sets exhibiting much higher differentiation levels. For example, in the wolf data used it appears that out of 36 pairwise F_{ST} values, only five were below 0.04, and 25 were larger than 0.058 (the average F_{ST} between the river sides) with values up to 0.188 (Carmichael *et al.* 2001). This explains the finer resolution obtained by Wilson & Rannala (2003).

These results are compatible with a model in which orang-utans move between neighbouring areas but do not cross the river, at least in the study area. In such a model, gene flow between the two river sides is maintained over generations through individuals crossing the river somewhere upstream. We cannot identify where orang-utans are most likely to cross the river, but in an isolation by distance model, the correlation between genetic and geographical distance increased when the crossing point was moved upstream until it reached values of 100–150 km. Put in a different way, the average F_{ST} observed between the river sides is equivalent to travelling approximately 200–300 km. Interestingly, these distances do correspond to regions where the river becomes narrower and crossing more plausible.

Previous studies on Borneo orang-utans have mostly used animals from rehabilitation centres. Warren *et al.* (2001) analysed mitochondrial DNA data from 41 individuals originating from six locations across Borneo including a sample from the Sepilok Orangutan sanctuary in Sabah. They found very large pairwise F_{ST} values between the samples (with two exceptions all values were larger than 0.48), and suggested that at least four biogeographical regions could be defined, namely (i) Southwest and Central Kalimantan, (ii) Northwest Kalimantan and Sarawak, (iii) Sabah, and (iv) East Kalimantan. Their study also suggested that the differentiation between these four regions could be very old (on the order of 860 000 years) and could therefore be a result of geographical barriers such as ancient river systems that separated populations during the colonization of the island from Sumatra. Our study confirms the potential role of rivers in isolating orang-utans at a much finer geographical scale. In an earlier study Warren *et al.* (2000), analysed orang-utans from East and West Kalimantan (the Indonesian part of Borneo) using five microsatellites with sample sizes between 10 and 43 individuals depending on the locus. They found that Nei's distance between East and West Kalimantan samples were small and had a large variance. They concluded that there was no significant differentiation at this scale. This result is at odds with both our results and those of Warren *et al.* (2001). One possible reason for the apparent discrepancy is that the conclusion of Warren *et al.* (2000) was based on the calculation of Nei's genetic distance and used small sample sizes. Comparison with our data is difficult, as they did not estimate F_{ST} values. For instance, their data indicated that diversity was higher within than between samples, which should not be interpreted as a lack of genetic differentiation and is indeed in agreement with our results (an F_{ST} of 0.02 indicates that 98% of the diversity is within sampled regions). We thus estimated F_{ST} values by using their Tables 1 and 2. The first provides allele frequencies and the second sample sizes for the different loci. Based on these tables we find that the single locus F_{ST}s are 0.061, 0.044, 0.004, 0.009 and −0.012 (i.e. 0.000). Thus, three loci essentially show no sign of

genetic differentiation and two show values similar to those observed across the river or between the most differentiated samples from RS2 (Fig. 2). It is difficult to make strong conclusions from these calculations, and we can only note that more loci and more samples would be needed to have a better understanding of genetic differentiation at wider geographical scales.

Overall, our results show that significant genetic differentiation exists among orang-utan groups separated by less than 200 km. Future studies should investigate the role played by human barriers such as oil palm plantations, riparian villages, or roads in the development of genetic differentiation between remaining forest patches. For instance, the Sandakan-Lahad Datu road is a very frequented road and may provide a significant barrier to current and future gene flow.

Some consequences for the conservation of orang-utans

In the present study we have shown that LK orang-utans have maintained relatively high levels of genetic variability despite the increasing fragmentation of their habitat. While this may be seen as good news for the conservation of orang-utans, some caution should be taken. The maintenance or increase of current population sizes, including gene flow (through translocation for instance), are required to mitigate against significant loss of genetic diversity. Our results suggest that orang-utans move rather freely between lots from the same side of the river and that little, if any, movement seems to take place across the Kinabatangan River in the study area. Current orang-utan populations may continue to decrease in many of the forest lots investigated even if forest fragmentation stops. For example, in some lots the number of individuals estimated to survive is already low, as in lot 8 (corresponding to S7, see Fig. 1) where Ancrenaz *et al*. (2004) estimated the census size to be approximately 22. In such lots, genetic drift is going to reduce genetic diversity very quickly. We simulated genetic drift in this lot and found that two alleles will be lost every three generations for the next 10 generations at least. Given that these simulations optimistically assume that the census size is equal to the effective size, the situation is likely to be much worse. There is therefore an urgent need to maintain, and even increase, migration between lots. This could be done, for instance, by restoring forest corridors alongside the river banks and between lots. Translocation between lots from opposite sides of the river may be feasible because the differentiation is limited and a number of individuals were assigned to the opposite river side. However, we believe that such translocations should be avoided until other regions are sampled both upstream and away from the Kinabatangan River. Indeed, whereas we cannot exclude that nonsampled 'populations' could account for these individuals, we have good reasons to think that orang-utans cannot cross the river in the area sampled. Of course, would population size keep decreasing, as could potentially happen in lot 8, translocation from any viable population would certainly be considered as a positive practical action. Whenever possible translocation between lots from the same side should be favoured. Moreover, wildlife surveys highlighted the importance of several areas in Sabah which need to be reconnected to each other (Ancrenaz *et al*. 2005). The time-frame for achieving corridor development may be hundreds of years, given the logistical challenges. Nonetheless, such systems are required if we are to conserve orang-utans and biodiversity in general in the long term.

Acknowledgements

We would like to thank the Economic Planning Unit and especially P Munirah for giving us permission to do research in Sabah. We also would like to thank SM Jensen and R Cox for their support to the project and to KOCP in general, C Andalo, J Setchell, M Schilthuizen, L Waits and two anonymous referees for useful comments on the manuscript, and B Crouau-Roy for support during the writing up of this work. We also thank G Wilson for discussions on BayesAss and for sending us the code, which M O'Hely helped us change. BG warmly thanks the KOCP research assistants and particularly JB Sinyor and ZB Abdullah, for their hard work and friendship during the sampling expeditions, and for climbing trees and collecting hairs in orang-utan nests. This work was funded by the Darwin Initiative for the Survival of Species (Grant no. 09/016, DEFRA, UK), Cardiff University, Universiti Malaysia Sabah, the HUTAN NGO. The writing of this paper was made possible thanks to a 7-month visiting position to BG funded by the Centre National pour la Recherche Scientifique (CNRS, France).

Orang-utan hair and faeces were exported to the UK under export permit from Federation of Malaysia (CITES Certificate no. 0467, security stamp No MY 9123707) and under import permit from the United Kingdom (CITES Certificates no. 236719/01 for shed hair samples and no. 236719/02 for faecal samples).

References

Ancrenaz M, Gimenez O, Ambu L *et al*. (2005) Aerial surveys give new estimates for orang-utans in Sabah, Malaysia. *PLos Biology*, **3**, xx.

Ancrenaz M, Goossens B, Gimenez O, Sawang A, Lackman-Ancrenaz I (2004) Determination of ape distribution and population size using ground and aerial surveys: a case study with orang-utans in Lower Kinabatangan, Sabah, Malaysia. *Animal Conservation*, **7**, 375–385.

Azmi R (1998) Natural vegetation of the Kinabatangan floodplain. Part 1: Background and preliminary checklist. *Report WWF-Malaysia*, Kota Kinabalu, Sabah.

Belkhir K, Borsa P, Chikhi L, Goudet J, Bonhomme F (1996/1997) *GENETIX 3.07, Windows™ Software for Population Genetics*. Laboratoire Génome et Populations, University of Montpellier II, Montpellier, France.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.

Brashares JS (2003) Ecological, behavioral, and life-history correlates of mammal extinctions in West Africa. *Conservation Biology*, **17**, 733–743.

Cardillo M (2003) Biological determinants of extinction risk: why are smaller species less vulnerable? *Animal Conservation*, **6**, 63–69.

Carmichael LE, Nagy JA, Larter NC, Strobeck C (2001) Prey specialization may influence patterns of gene flow in wolves of the Canadian Northwest. *Molecular Ecology*, **10**, 2787–2798.

Cegelski CC, Waits LP, Anderson NJ (2003) Assessing population structure and gene flow in Montana wolverines (*Gulo gulo*) using assignment-based approaches. *Molecular Ecology*, **12**, 2907–2918.

Chikhi L, Bruford MW (2005) Mammalian population genetics and genomics. In: *Mammalian Genomics* (eds Ruvinsky A, Marshall Graves J), pp. 539–584. CABI Publishers, UK.

Chikhi L, Bruford MW, Beaumont MA (2001) Estimation of admixture proportions: a likelihood-based approach using Markov chain Monte Carlo. *Genetics*, **158**, 1347–1362.

Coote T, Bruford MW (1996) Human microsatellites applicable for analysis of genetic variation in Apes and Old World monkeys. *Journal of Heredity*, **87**, 406–410.

Cornuet J, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics*, **153**, 1989–2000.

Davies KF, Margules CR, Lawrence KF (2000) Which traits of species predict population declines in experimental forest fragments? *Ecology*, **81**, 1450–1461.

Delgado RA, van Schaik CP (2000) The behavioral ecology and conservation of the orangutan (*Pongo pygmaeus*): a tale of two islands. *Evolutionary Anthropology*, **9**, 201–218.

Ellegren H, Moore S, Robinson N *et al.* (1997) Microsatellite evolution — a reciprocal study of repeat lengths at homologous loci in cattle and sheep. *Molecular Biology and Evolution*, **14**, 854–860.

Galdikas GMF (1995) Social and reproductive behavior of wild adolescent female orangutans. In: *The Neglected Ape* (eds Nadler RD, Galdikas BMF, Sheeran LK, Rosen N), pp. 163–182. Plenum Press, New York, USA.

Gilks WR, Richarson S, Spiegelhalter DJ (1996) *Markov Chain Monte Carlo in Practice*. Chapman & Hall, New York.

Goossens B, Abdullah ZB, Sinyor JB, Ancrenaz M (2004) Which nests to choose: collecting shed hairs in wild orang-utans. *Folia Primatologica*, **75**, 23–26.

Goossens B, Chikhi L, Ancrenaz M, Lackman-Ancrenaz I, Andau M, Bruford MW (submitted) Genetic signature of anthropogenic population collapse in. orang-utans.

Goossens B, Chikhi L, Utami SS, de Ruiter J, Bruford MW (2000a) A multi-samples, multi-extracts approach for microsatellite analysis of faecal samples in an arboreal ape. *Conservation Genetics*, **1**, 157–162.

Goossens B, Funk SM, Vidal C *et al.* (2002) Measuring genetic diversity in translocation progammes: principles and application to a chimpanzees release project. *Animal Conservation*, **5**, 225–236.

Goossens B, Latour S, Vidal C *et al.* (2000b) Twenty new microsatellite loci for use with hair and faecal samples in the chimpanzee (*Pan troglodytes troglodytes*). *Folia Primatologica*, **71**, 177–180.

Harcourt AH (2002) Empirical estimates of minimum viable population sizes for primates: tens to tens of thousands? *Animal Conservation*, **5**, 237–244.

Keller LF, Jeffery KJ, Arcese P *et al.* (2001) Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. *Proceedings of the Royal Society London*, **268**, 1387–1394.

Kinnaird MF, Sanderson EW, O'Brien TG, Wibisono HT, Woolmer G (2003) Deforestation trends in a tropical landscape and implications for endangered large mammals. *Conservation Biology*, **17**, 245–257.

Lackman-Ancrenaz I, Ancrenaz M, Saburi R (2001) The Kinabatangan Orang-utan Conservation Project (KOCP). In: *Proceedings of a Conference on the Apes: Challenges for the 21st Century* (ed. Chicago Zoological Society), pp. 262–265. Brookfield Zoo, Illinois, USA.

Laidlaw RK (2000) Effects of habitat disturbance and protected areas on mammals of Peninsular Malaysia. *Conservation Biology*, **14**, 1639–1648.

Laurance WF (1991) Ecological correlates of extinction proneness in Australian tropical rainforest mammals. *Conservation Biology*, **5**, 79–89.

Leendertz FH, Ellerbrok H, Boesch C *et al.* (2004) Anthrax kills wild chimpanzees in a tropical rainforest. *Nature*, **430**, 451–452.

Leighton M, Seal US, Soemarna K *et al.* (1995) Orangutan life history and vortex analysis. In: *The Neglected Ape* (ed. Nadler RD, Galdikas BMF, Sheeran LK, Rosen N), pp. 97–108. Plenum Press, New York, USA.

Leroy EM, Rouquet P, Formenty P *et al.* (2004) Multiple ebola virus transmission events and rapid decline of Central African wildlife. *Science*, **303**, 387–390.

Madsen T, Shine R, Olsson M, Wittzell H (1999) Conservation biology — Restoration of an inbred adder population. *Nature*, **402**, 34–35.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

McMorrow J, Talip MA (2001) Decline of forest area in Sabah, Malaysia: relationship to state policies, land code and land capability. *Global Environmental Change*, **11**, 217–230.

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): implications for conservation. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 4334–4339.

Mitani JC (1989) Orangutan activity budgets: monthly variations and the effects of body size, parturition, and sociality. *International Journal of Primatology*, **18**, 87–100.

Möller LM, Beheregaray LB (2004) Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). *Molecular Ecology*, **13**, 1607–1612.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. *Evolution*, **29**, 1–10.

O'Ryan C, Harley EH, Bruford MW *et al.* (1998) Microsatellite analysis of genetic diversity in fragmented South African buffalo populations. *Animal Conservation*, **1**, 124–131.

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetic Research*, **22**, 201–204.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Palsson S (2004) Isolation by distance, based on microsatellite data, tested with spatial autocorrelation (SPAIDA) and assignment test (SPASSIGN). *Molecular Ecology Notes*, **4**, 143–145.

Payne J (1987) Surveying orang-utan populations by counting nests from a helicopter; a pilot survey in Sabah. *Primate Conservation*, **8**, 92–103.

Payne J (1988) *Orang-utan Conservation in Sabah*. WWF Malaysia, Kuala Lumpur.

Pryor KV, Young JE, Rumsey FJ *et al.* (2001) Diversity, genetic structure and evidence of outcrossing in British populations of the rock fern *Adiantum capillus-veneris* using microsatellites. *Molecular Ecology*, **10**, 1881–1894.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 9197–9201.

Reed DH (2004) Extinction risk in fragmented habitats. *Animal Conservation*, **7**, 181–191.

Rijksen HD, Meijaard E (1999) *Our Vanishing Relative: the Status of Wild Orang-utans at the Close of the 20th Century*. Kluwer Academic Publishers, Dordrecht.

Robertson JMY, van Schaik CP (2001) Causal factors underlying the dramatic decline of the Sumatran orang-utan. *Oryx*, **35**, 26–38.

Rodman PS, Mitani JC (1987) Orangutans: sexual dimorphism in a solitary species. In: *Primate Societies* (eds Smuts B, Cheney DL, Seyfarth RM, Struhsaker T, Wrangham RW), pp. 146–154. University of Chicago Press, Chicago, USA.

Saccheri I, Kuussaari M, Kankare M *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

van Schaik CS, Monk KA, Robertson JMY (2001) Dramatic decline in orang-utan numbers in the Leaser Ecosystem, Northern Sumatra, *Oryx*, **35**, 14–25.

Singleton I, van Schaik CP (2001) Orangutan home range size and its determinants in a Sumatran swamp forest. *International Journal of Primatology*, **22**, 877–911.

Taberlet P, Griffin S, Goossens B *et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, **24**, 3189–3194.

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, **14**, 323–327.

Travis JMJ, Dytham C (1999) Habitat persistence, habitat availability and the evolution of dispersal. *Proceedings of the Royal Society London*, **266**, 723–728.

Utami SS, Goossens B, Bruford MW, de Ruiter J, van Hooff JARAM (2002) Male bimaturism and reproductive success in Sumatran orang-utans. *Behavioral Ecology*, **13**, 643–652.

Vigilant L (1999) An evaluation of techniques for the extraction and amplification of DNA from naturally shed hairs. *Biological Chemistry*, **380**, 1329–1331.

Walsh PD, Abernethy KA, Bermejo M *et al.* (2003) Catastrophic ape decline in western equatorial Africa. *Nature*, **422**, 611–614.

Warren KS, Nijman IJ, Lenstra JA *et al.* (2000) Microsatellite DNA variation in Bornean orang-utans (*Pongo pygmaeus*). *Journal of Medical Primatology*, **29**, 57–62.

Warren KS, Verschoor EJ, Langenhuizen S *et al.* (2001) Speciation and intrasubspecific variation of Bornean orang-utans, *Pongo pygmaeus pygmaeus*. *Molecular Biology and Evolution*, **18**, 472–480.

Webb JK, Brook BW, Shine R (2002) What makes a species vulnerable to extinction? Comparative life-history traits of two sympatric snakes. *Ecological Research*, **17**, 59–67.

Weir BS (1979) Inferences about linkage disequilibrium. *Biometrics*, **35**, 235–254.

Weir BS, Cockerham CC (1984) Estimating *F* statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wich SA, Singleton I, Utami-Atmoko SS *et al.* (2003) The status of the Sumatran orang-utan *Pongo abelii*: an update. *Oryx*, **37**, 49–54.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **163**, 1177–1191.

Zhang YW, Morin PA, Ryder OA, Zhang YP (2001) A set of human tri- and tetra-nucleotide microsatellite loci useful for population analyses in gorillas (*Gorilla gorilla gorilla*) and orang-utans (*Pongo pygmaeus*). *Conservation Genetics*, **2**, 391–395.

Zhi L, Karesh WB, Janczewski DN *et al.* (1996) Genomic differentiation among natural populations of orang-utan (*Pongo pygmaeus*). *Current Biology*, **6**, 326–336.

B Goossens is a research associate at Cardiff University working on the population genetic structure, mating system and conservation genetics in a number of endangered mammal species. L Chikhi is a researcher at the 'Evolution et Diversité Biologique' CNRS Group in Toulouse, interested in using molecular markers to infer ancient population demography, with a particular emphasis on humans and primates. MF Jalil is a Malaysian PhD student at Cardiff University working on a comparative phylogeography of three primate species in Sabah. M Ancrenaz and I Lackman-Ancrenaz are the Directors of the KOCP based in the Kinabatangan flood plain. M Mohamed is the Director of the Institute for Tropical Biology and Conservation at Universiti Malaysia Sabah and is interested in entomological biodiversity and ecology. P Andau is the Director of the Sabah Wildlife Department. MW Bruford is interested in the evolutionary ecology and population genetics of threatened species.

Appendix 1

Tests of linkage disequilibrium (LD). LD was measured using the correlation coefficient (Weir 1979). We represent the proportion of randomised values greater or equal to the observed correlation coefficient

Locus 1	Locus 2	S1	S2	S3	S4	S5	S6	S7	S8	S9
D5S1457	D5S1470	0.9	76.6	92.9	39.5	98.1	19.4	3.1	24.2	27.1
D5S1457	D1S550	43.5	58.4	7.5	73.7	38.7	29.1	14.1	87.1	65.4
D5S1457	D2S1326	47	4.6	35.4	88	85.3	78.6	100	39.5	5
D5S1457	D3S2459	49.4	16.9	0	53.8	54.6	66.9	61.3	72.6	0
D5S1457	D4S1627	35.9	31	22.5	40.1	72.6	53.8	71	32.3	84.8
D5S1457	D4S2408	6	60.1	4.4	51.2	18.3	22.8	100	0.3	16.2
D5S1457	D5S1505	84.6	89.9	99.8	4.9	81.8	63.6	20.9	2.6	45.5
D5S1457	D6S501	4.8	58.4	9.6	49.8	4.4	13.5	10.2	88.8	21.8
D5S1457	D13S321	11.5	1.8	5.6	75.1	54.9	60.5	22.1	65	30.7
D5S1457	D13S765	77.1	31.2	16.8	78.9	9.4	32.9	37.9	19.7	11.2
D5S1457	D12S375	3	51.5	44.7	0.5	59.5	71.8	63.9	64	73.6
D5S1457	D2S141	4.6	76.4	33.1	20.7	51.1	28.5	62.5	39.2	92.3
D5S1457	D16S420	29.4	22.8	11.7	73.4	78.5	29.4	12.6	45.4	35.8
D5S1470	D1S550	37.9	73	30.1	1.7	32.6	83	6.7	42.5	74
D5S1470	D2S1326	28	36.1	4	28	49.6	90.5	41.2	98.5	52.3
D5S1470	D3S2459	82.3	0.6	51.9	12.2	52.5	85.4	57.7	58.7	22
D5S1470	D4S1627	0.1	63.6	18	76	32.9	86.2	20	93	87.4
D5S1470	D4S2408	0.2	42.6	79.2	2.7	96.6	8.2	100	4.8	28.4
D5S1470	D5S1505	8.2	96.7	17.3	88.2	3.8	68.1	19.1	1.5	10
D5S1470	D6S501	16.9	3.9	22.4	74.9	17.1	12.6	52.9	98.2	3
D5S1470	D13S321	62.7	30.5	24.1	68.3	51.6	16.3	20	72.1	87.9
D5S1470	D13S765	57	20.6	76.6	64.2	40.2	55.2	79.5	88.8	10.5
D5S1470	D12S375	17.8	89	17.1	56.6	71.3	99.1	6.9	11.6	64.9
D5S1470	D2S141	3.3	3.4	50.2	46.5	13.2	95.2	22.5	33.9	90.9
D5S1470	D16S420	30.7	80.9	63.3	0.2	76.5	52.4	2.7	85.7	33.9
D1S550	D2S1326	85.7	91.2	17.4	57.9	36.3	56.3	89.8	40.8	63.8
D1S550	D3S2459	81.6	6.2	35.9	87.1	71.5	42.1	49.4	42.1	55.4
D1S550	D4S1627	18.2	54.7	17.1	91.8	74.2	80.7	38.4	74.2	75.9
D1S550	D4S2408	46.3	49.1	32.3	0.6	43	5.1	79.6	52.2	60
D1S550	D5S1505	38.3	7	1.6	88.6	30.8	25.4	32	4.8	50.5
D1S550	D6S501	92.5	34.1	16.6	8	2.4	1.8	43.9	46.9	48.6
D1S550	D13S321	53.4	14.6	1.5	12.4	55.9	7.9	61.2	70.7	62.7
D1S550	D13S765	3.4	86.6	2.3	67.7	95.8	77.7	60.3	79.4	36.9
D1S550	D12S375	39.2	52.8	86.9	22.6	15.7	41.3	12.3	71.2	46.1
D1S550	D2S141	42.3	8.9	58	37.3	7.7	6.7	28.8	82.8	22.4
D1S550	D16S420	0.2	73.1	68.5	54.6	28.6	0	8.9	94.9	84.6
D2S1326	D3S2459	21.6	21	0	36.6	15.3	36.6	10.2	64.1	14.5
D2S1326	D4S1627	41.5	51.5	2.8	35.2	1.3	61	19	26.6	83.9
D2S1326	D4S2408	3.7	80.5	28.7	51.9	2.4	65.7	40	59.3	91.9
D2S1326	D5S1505	18.3	50.9	2.8	9.2	71.6	2.4	37.5	47.7	57.5
D2S1326	D6S501	50.4	83.9	1.9	20.3	12.3	5.5	9.4	1	3.2
D2S1326	D13S321	84.2	23.5	4.8	15.1	86.1	72.7	25.1	39.6	9.7
D2S1326	D13S765	13.5	9.4	8.2	75.5	43.9	40.5	10.3	51.1	20.1
D2S1326	D12S375	10.9	72	0.3	28.9	99.3	75.6	60.6	18.2	9.9
D2S1326	D2S141	3.4	56	42.1	73.7	40.9	67.1	27.5	36.5	96.2
D2S1326	D16S420	59.4	0.3	0.9	5.6	65.7	7.6	9.8	64.5	16.2
D3S2459	D4S1627	51.2	72.7	4.9	13.6	0.4	45.1	90.4	2.5	9.2
D3S2459	D4S2408	93	47.9	0.4	71.2	0.1	61.3	100	54.9	0.2
D3S2459	D5S1505	69	71.6	23.2	18.3	27.4	65.9	50.1	56.8	19.5
D3S2459	D6S501	0.9	54.5	6.6	3	8	81.7	39.5	12.9	22.3
D3S2459	D13S321	21.9	69.3	1.9	55.5	80.4	50.5	42.1	57.7	39.9
D3S2459	D13S765	77.2	33.3	5.9	58.9	18.8	30.6	27.8	75.3	37.4
D3S2459	D12S375	55.8	69.6	3.8	27.4	14	83.5	27.3	76.3	34.7
D3S2459	D2S141	35.8	17.2	27.9	32	58.4	0.8	71.1	88.1	78.1
D3S2459	D16S420	61.3	78.1	3.7	13.2	74.7	52.6	5.7	87.9	36.2

Appendix 1 *Continued*

Locus 1	Locus 2	S1	S2	S3	S4	S5	S6	S7	S8	S9
D4S1627	D4S2408	0	19.3	34.8	26.7	25.3	13.7	39.2	29.5	54.3
D4S1627	D5S1505	87.2	2.1	44.8	78.5	16.2	67.6	14.6	10.4	48.8
D4S1627	D6S501	7.4	36.8	52	30.5	31.4	80.7	46.5	3.5	20.2
D4S1627	D13S321	29.6	12.4	8	36.4	24	39.5	5.4	4.5	92
D4S1627	D13S765	0.2	14.3	64.4	45.9	75.1	49.2	100	73.2	88.9
D4S1627	D12S375	21.4	76	0.7	7.8	56.5	9.3	49.9	97.6	21
D4S1627	D2S141	35.5	42.5	11.1	0.8	92	18.9	7.6	5.2	12.5
D4S1627	D16S420	30.7	94.8	35.6	95.7	56.4	51	11.3	15.8	6
D4S2408	D5S1505	33.9	27.9	80.8	24	91.2	71	100	1.9	19.6
D4S2408	D6S501	16.6	91.6	16.3	7.4	6.8	40.5	100	51.9	58.7
D4S2408	D13S321	3.8	21.6	2.8	89.4	68.7	13.8	100	21.7	47.3
D4S2408	D13S765	11.9	42.8	12.3	80.6	12.9	14.7	39.9	18.6	36.8
D4S2408	D12S375	65.1	64.2	5.7	1.7	52.1	23.1	42.3	76.6	54.7
D4S2408	D2S141	78.3	19	21.1	38.5	39.2	37.9	79.1	6.1	69.6
D4S2408	D16S420	7.4	6	41.8	55.8	85.5	10	60.4	23.8	42.9
D5S1505	D6S501	1.7	81.8	12.2	23.1	14.5	93.2	21.4	78.4	74.3
D5S1505	D13S321	22.4	45.2	2.5	83.4	85.5	82.2	9.4	10.7	52.6
D5S1505	D13S765	84.6	29.4	6.2	70	58.7	25.4	72	65.2	91.5
D5S1505	D12S375	34.8	23.4	86.6	14.2	95.3	64.2	100	21.8	45.4
D5S1505	D2S141	5	4.9	56.6	80.9	57.9	99.5	15	18.8	46.9
D5S1505	D16S420	54.1	16	9.7	3.5	30.5	72.1	30.5	7.5	41.5
D6S501	D13S321	7.8	47	6.2	33.8	78.6	59.7	20.6	20.7	75.5
D6S501	D13S765	14.9	39.1	1.3	79.8	0.1	4	31.4	61.4	15.8
D6S501	D12S375	47.1	72.3	8.4	75.6	57.7	36.3	63.3	87.5	51.1
D6S501	D2S141	27.1	81.6	6.4	90.4	36.9	50.2	20.4	82	56.4
D6S501	D16S420	90.8	60.7	0.4	33.7	68.6	9.4	31.7	60.7	77.3
D13S321	D13S765	66.2	78.4	14	18.2	13.5	61.2	100	20.5	58.7
D13S321	D12S375	26.3	78.8	18.7	35	28.1	26.4	100	22.3	3.8
D13S321	D2S141	14.6	75.8	6.5	36	49.2	47.2	18.4	68.2	0.7
D13S321	D16S420	23.3	18.6	39.1	32.1	33.7	57.5	28.9	6.5	41.6
D13S765	D12S375	76	55.4	65.8	67.8	51.8	19.1	18.7	26.4	34
D13S765	D2S141	50	4.6	98.4	62.7	68.2	50.8	78.5	35.5	95.8
D13S765	D16S420	29.7	5.7	31.2	85.1	84.6	69.9	19.9	56.9	44.1
D12S375	D2S141	1.7	32.4	4.6	11.3	12.1	44.5	79.6	24.6	47.4
D12S375	D16S420	35	12	70.6	1.6	1.9	88.1	16	75	21.1
D2S141	D16S420	6.5	0.8	22.7	41.6	0.4	4.8	30.7	10.3	55