MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 3352–3362 doi: 10.1111/j.1365-294X.2012.05608.x

Parentage-based pedigree reconstruction reveals female matrilineal clusters and male-biased dispersal in nongregarious Asian great apes, the Bornean orang-utans (*Pongo pygmaeus*)

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Abstract

Philopatry and sex-biased dispersal have a strong influence on population genetic structure, so the study of species dispersal patterns and evolutionary mechanisms shaping them are of great interest. Particularly nongregarious mammalian species present an underexplored field of study: despite their lower levels of sociality compared to group-living species, interactions among individuals do occur, providing opportunities for cryptic kin selection. Among the least gregarious primates are orang-utans (genus: *Pongo*), in which preferential associations among females have nevertheless been observed, but for which the presence of kin structures was so far unresolved because of the equivocal results of previous genetic studies. To clarify relatedness and dispersal patterns in orang-utans, we examined the largest longitudinal set of individuals with combined genetic, spatial and behavioural data. We found that males had significantly higher mitochondrial DNA (mtDNA) variation and more unique haplotypes, thus underscoring their different maternal ancestries compared to females. Moreover, pedigree reconstruction based on 24 highly polymorphic microsatellite markers and mtDNA haplotypes demonstrated the presence of three matrilineal clusters of generally highly related females with substantially overlapping ranges. In orang-utans and possibly other nongregarious species, comparing average biparental relatedness (*r*) of males and females to infer sex-biased dispersal is extremely problematic. This is because the opportunistic sampling regime frequently employed in nongregarious species, combined with overlapping space use of distinct matrilineal clusters, leads to a strong downward bias when mtDNA lineage membership is ignored. Thus, in nongregarious species, correct inferences of dispersal can only be achieved by combining several genetic approaches with detailed spatial information.

Keywords: kin structure, matrilineal cluster, nongroup-living species, relatedness

Received 20 December 2011; revision accepted 19 March 2012

Introduction

Sex-biased natal dispersal, whereby one sex displays a greater tendency to leave or travel longer distances away from the natal area before breeding, is ubiquitous in the animal kingdom (Howard 1960; Clobert *et al.* 2001). This crucial life history trait has a strong impact on population genetic structure, influencing the maintenance and loss of genetic diversity in populations (Chesser 1991b; Sugg *et al.* 1996; Storz 1999). Hence,

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resolving a species' dispersal pattern as well as the mechanisms that drive these is of great interest.

Some of the evolutionary mechanisms invoked to explain the tendency for one sex to exhibit site fidelity or philopatry, i.e. the tendency to breed within or in close proximity to the natal range, include ecological benefits. For instance, philopatric individuals might benefit from familiarity with resources and avoid the risks associated with migration through unknown areas (Greenwood 1980; Lawson Handley & Perrin 2007). Philopatry results in kin structures that might also confer social benefits because of nepotistic interactions, providing inclusive fitness benefits that could augment or even drive philopatry (Perrin & Goudet 2001; Lawson Handley & Perrin 2007). The prediction for species with the mate-defence mating systems prevalent among mammals is that females, who benefit most from acquaintance with a given territory, should be philopatric, with males dispersing to avoid kin competition and inbreeding (Greenwood 1980; Dobson 1982; Pusey 1987; Wolff 1993).

The social organization of group-living mammals has drawn particularly intense interest. In these species, the salient social interactions have prompted many genetic studies to investigate whether kin structures among same-sex members underlie social behaviours such as tolerance, cooperation, learning and cultural variation (spotted hyenas; Van Horn *et al.* 2004; chimpanzees, Lukas *et al.* 2005; horses; Cameron *et al.* 2009; chacma baboons, King *et al.* 2011). Far fewer studies, however, have examined relatedness patterns in nongregarious species. Nevertheless, individuals of nongregarious species may have 'social networks' (Charles-Dominique 1978), engaging in associations with neighbours, so opportunities for cryptic kin selection to operate exist (Hatchwell 2010). Consequently, the exploration of kin structures in such species may lead to important new insights.

The few genetic investigations to date of nongregarious mammals have concentrated on carnivores (raccoons, Ratnayeke *et al.* 2002; cougars, Biek *et al.* 2006; bears, Zedrosser *et al.* 2007) and rodents (woodrats, McEachern *et al.* 2007), as well as a few lemur species among the primates (Kappeler *et al.* 2002; Eberle & Kappeler 2006; Radespiel *et al.* 2009). Such studies have proven invaluable, as illustrated by the examination of the solitarily foraging grey mouse lemur, a species in which females allo-nurse in diurnal sleeping groups. The usage of genetic markers enabled Eberle & Kappeler (2006) to establish that allo-nursing females comprised close maternal relatives, thus providing strong evidence for kin-based communal breeding. In other species without such opportunities for association, nepotistic behaviour could nonetheless still occur albeit in less obvious ways, for instance through reduced aggression and increased tolerance towards relatives that might make settlement in familiar areas easier (Perrin & Goudet 2001; Hatchwell 2010).

Among the most enigmatic nongregarious species are the Asian great apes, the orang-utans (genus: *Pongo*). Like most other great apes, orang-utans have a fission–fusion social system. But they stand out as a result of their especially low levels of sociality (van Schaik 1999) and possibly different social organization. In orang-utans, behavioural evidence points to female philopatry and male-biased dispersal (Galdikas 1985b; Mitani 1989; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000), while in African great apes and humans, female dispersal is common (Eriksson *et al.* 2006; Wilkins & Marlowe 2006; Douadi *et al.* 2007; Langergraber *et al.* 2007; Guschanski *et al.* 2008). Such a dispersal pattern might affect associations among individuals, which despite occurring infrequently, do take place (van Schaik 1999; Delgado & Van Schaik 2000).

Yet the pattern of sex-biased dispersal in orang-utan populations is not clear. Broad-scale studies show tighter geographical clustering of mtDNA compared to Y-chromosome haplotypes across the highly differentiated orang-utan populations (Arora *et al.* 2010; Nietlisbach *et al.* accepted), suggesting historical male-mediated gene flow. Nevertheless, three previously published local scale studies of contemporary dispersal examining relatedness within populations did not confirm this pattern. These studies were based on conventional genetic methodology relying on the comparison of average pairwise relatedness (r) estimates of adult females and adult males obtained using biparentally inherited microsatellite markers. The expectation is that the more philopatric sex comprising related individuals should have higher r values than the dispersing sex comprising immigrants (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007). The relatedness comparisons of the three studies were indicative of dispersal of both sexes (Utami *et al.* 2002), philopatry of both sexes (Goossens *et al.* 2006) or male-biased dispersal (Morrogh-Bernard *et al.* 2011). Nevertheless, the inclusion of rehabilitants in the first study, habitat fragmentation in the second study and the smaller sample size in the third study might have been responsible for these differences. The discrepant behavioural and genetic results render the social organization of orang-utans unresolved. It is also unclear whether contemporary dispersal patterns are at odds with historical patterns. Determining whether orang-utans have kin structures and how these are linked to dispersal is crucial step before investigating the possible evolutionary mechanisms underlying the movement of individuals and genes, population genetic structure, and social behaviour.

The aim of the present study was to gain an insight into the dispersal and relatedness patterns of orang-utans, based on the ongoing long-term study at Tuanan Orang-utan Research Area, Borneo, Indonesia. We capitalized on the largest set of genetically characterized sexually mature individuals ($n = 40$) from a natural population of orang-utans to test genetic predictions based on field observations of female philopatry and male-biased dispersal. We included only sexually mature individuals because they have potentially already settled within the natal area or dispersed to breed (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007). By complementing spatial and behavioural information, as well as genetic data from the maternally inherited mitochondrial DNA (mtDNA) and a panel of 24 autosomal microsatellite markers, we tested the following predictions:

1. *MtDNA diversity patterns.* Diversity levels are expected to be higher for males if they are the dispersing sex, reflecting their more varied maternal ancestries.
2. *Pedigree relationships.* The number of closely related dyads, and especially maternally related dyads, as estimated from a parentage-based pedigree reconstruction, is expected to be higher among females compared to males.
3. *Average pairwise relatedness estimates.* The estimates are expected to be higher among females than males, as the latter should comprise immigrants.

In addition to disentangling the dispersal patterns in orang-utans, we discuss the effects of sampling regime, life history traits and spatial distribution of individuals on relatedness estimation, which is especially significant when studying nongroup-living animals.

Materials and methods

Study population

Sampling was conducted in the Tuanan Orang-utan Research Area (2°09′ South; 114°26′ East), Mawas Conservation Area, Central Kalimantan, Indonesia. This site is located within a peat swamp forest of approximately 750 ha, accessible through grid-based trails. The orang-utan density estimate for the area is 4.25–4.5 individuals per km^2 (van Schaik *et al.* 2005). Females at this site have home ranges estimated to be 325 ha (±125 ha) (Wartmann *et al.* 2010; van Noordwijk *et al.* 2012). Among males, two morphs are found: flanged males, which have fully developed irreversible secondary sexual characteristics, and unflanged males, which have not (Delgado & Van Schaik 2000; Utami *et al.* 2002). Home ranges of both flanged and unflanged males are far larger than those of females, also exceeding the size of the study site; their sizes are, therefore, unknown (Utami Atmoko *et al.* 2009; van Noordwijk *et al.* 2012).

Behavioural, spatial and genetic data collection

At this longitudinal study site, an intensive sampling regime from 2003 to 2009 targeted the collection of combined behavioural, spatial and genetic data for each individual, following the standard orang-utan protocol (http://www.aim.uzh.ch/orangutannetwork/FieldGuidelines.html). Trained observers conducted over 25 000 h of focal follows, normally nest-to-nest, to record behavioural and spatial information including space use, frequency of sightings, sex and age (Wich *et al.* 2004; van Noordwijk *et al.* 2012). The age of individuals born after 2003 was either known or estimated to the closest year; for individuals born before 2003, age was estimated based on known landmark ages in orang-utans (Wich *et al.* 2004).

Faecal samples were obtained during focal follows of individuals. Multiple samples were collected per individual throughout the study period and throughout the entire study area. We extracted DNA from the faecal samples with the QIAamp DNA Stool Mini Kit (Qiagen) and followed the manufacturer's protocol with a slight modification: elution was preceded by a 30-min incubation period. We genotyped individuals at up to 24 autosomal microsatellite markers and sequenced 450 bp of the hypervariable region I (HVRI) of the mtDNA using the same procedures as described in Arora *et al.* (2010).

For the genotyping, we minimized the genotyping errors associated with low quantity and quality of DNA obtained from noninvasively collected samples through the approach established by Morin *et al.* (2001). This method involves DNA quantification in each extract through real-time quantitative polymerase chain reaction (rtPCR), so as to determine the number of positive PCR replicates required to achieve a 99% certainty in a homozygous genotype. For a heterozygous genotype, the observation of each of the two alleles at least twice in independent PCRs is required. We initially used a panel of six autosomal microsatellite markers to genotype all samples obtained from potentially distinct individuals (Table S1, Supporting information). These markers were chosen because of their low-cumulative nonexclusion probabilities: 1.36×10^{-5} for unrelated individuals and 8.90×10^{-3} for full siblings, as determined by Cervus 3.0 (Kalinowski *et al.* 2007). Usage of these markers allowed us to distinguish unique individuals, providing a genetic method to link the behaviour of followed individuals to their genetic identity in a

longitudinal study. When repeated genotypes were obtained, we discarded all but one to have a data set of distinct individuals. These unique individuals were further genotyped at an additional 18 loci, resulting in a total of 24 autosomal microsatellite markers (Table S1), which were all in Hardy–Weinberg equilibrium, and showed no evidence of linkage disequilibrium or null alleles, as tested using Arlequin 3.11 (Excoffier *et al.* 2005), GenePop 4.0 (Rousset 2008) and ML-NullFreq (Kalinowski & Taper 2006), respectively. Details on the primers and PCR amplification conditions are described in the supporting information. For seven adult males, low autosomal DNA quality and quantity allowed only partial genotypes, restricted to the six markers used in the identity analyses. In total, multi-locus autosomal genotypes were obtained for 19 females and 29 males.

To obtain haplotype information, we sequenced 450 bp of the hypervariable region I (HVRI) of the mtDNA. Details on the primers, PCR amplification and raw data analyses are given in the Supporting information. MtDNA haplotypes were available for all genotyped individuals as well as one additional male with a unique mtDNA haplotype but no autosomal genotype.

Statistical analyses

We carried out the following analyses: (i) mtDNA diversity patterns, (ii) spatial distribution of females, whose ranging can be followed, (iii) parentage-based pedigree reconstruction and (iv) relatedness estimates. Unless specified otherwise, the analyses included only adult individuals who had potentially already settled within the natal area or dispersed to breed (Prugnolle & de Meeus 2002; Lawson Handley & Perrin 2007), the potential postdispersal (PPD) individuals. We considered individuals as PPD if they were sexually mature and/or regularly seen to range independently from the mother from the beginning of the study period (i.e. ranging at more than 50 m distance for at least several consecutive days). Individuals maturing during the study period were not included as PPD. These criteria resulted in a total of 40 PPD individuals ($n_{females}$ = 15; n_{males} = 25). The number of individuals included in each of the analyses detailed later is summarized in Table S3.

MtDNA diversity patterns and spatial distribution. Using the HVRI haplotypes, we conducted several analyses to assess patterns of mtDNA diversity and lineage relatedness. First, we compared levels of nucleotide and haplotype diversity for the PPD females and males using DNAsp v.5.0 (Librado & Rozas 2009). We tested for a significant difference in haplotype diversity between the sexes using a randomization test. For this, we randomly assigned all observed haplotypes to all males and females 1000 times and counted the number of instances in which the difference between male and female haplotype diversity exceeded the observed one. To show the mutational distances between the haplotypes found in the population as well as their frequencies according to sex, we generated a median-joining network using Network v4.6 and Network Publisher v1.2.0 (Bandelt *et al.* 1999; http://www.fluxus-engineering.com). Second, we assigned individuals to mtDNA lineages, defining these on the basis of haplotype sharing, irrespective of the biparental kinship of individuals.

For the PPD females, we also investigated the spatial distribution of mtDNA lineages using ArcGIS v.9.3.1 (ESRI 2008). To illustrate the areas within the study site where females with the same mtDNA haplotype, i.e. mtDNA lineages, were observed, we used the HRT plug-in for ArcGIS (Rodgers *et al.* 2007) to calculate 95% kernel probability plots, aggregating spatial data for all females with the same haplotype. Hence, incomplete ranging data for the females who also frequently moved outside of the study area did not affect the analyses. Spatial data were available for 13 PPD females (see Supporting information).

Parentage-based pedigree analyses. We examined the precise genetic relationships of female–female, male–male and female–male dyads through a combination of parentage and mtDNA analyses. First, we used the likelihood-based approach as implemented in Cervus 3.0 (Kalinowski *et al.* 2007) to carry out a parentage analysis for all PPD individuals for which data for 24 microsatellite markers were available ($n_{females}$ = 15; n_{males} = 17), as well as nine dependent offspring (see Supporting information).

Simulations were conducted to determine critical values of the log-likelihood score for a 95% confidence parentage assignment. The parameters for these simulations were 10,000 cycles and a minimum of 10 loci typed. The specified genotyping error rate of 0.112% was determined through the 'repeat-genotyping' and 'unintentionally re-sampled individuals' approaches described by Hoffman & Amos (2005). Only PPDs were incorporated as candidate mothers or fathers. The proportion of candidate parents was difficult to estimate from field data. Given the large influence this may have on the statistical significance of the results (Krützen *et al.* 2004a), several conservative values for this parameter (0.05, 0.08 and 0.10) were tested to check the robusticity of assignments. To examine the genetic relationships among all individuals including the seven additional males for which only a panel of six microsatellite markers was available, we repeated the parentage analyses with the same parameters, but with a specification of a minimum of five loci typed.

Following the parentage assignments, we inferred maternal and paternal sibling relationships by examining the shared mothers and shared fathers for each individual in the data set (n = 48). Such a parentage-based pedigree reconstruction allowed assessment of the number of maternal and paternal relatives at the site for each individual, incorporating parent-offspring and sibling relationships. These numbers represent only a minimum bound because the inference of genealogical relationships requires assignment to a parent and hence sampling of this parent within the study site, which may be limited by factors including emigration or death.

Relatedness analyses. We estimated average pairwise relatedness (r) coefficients for all PPD males and females in the data set. Two analyses were carried out. First, r was estimated for all same-sex individuals. Second, r was estimated for each set of same-sex individuals sharing their mtDNA haplotype.

To calculate r estimates, we used the triadic likelihood estimator (TrioML; Wang 2007). This estimator computes relatedness of a dyad in relation to a third reference individual in order to reduce errors stemming from identity-in-state rather than identity-by-descent. It further allows the specification of a genotyping error rate and is bounded between 0 and 1, a more legitimate range than that of other estimators. Moreover, an evaluation using empirical and simulated data for seven different estimators showed that the TrioML produced overall the most accurate estimates (Wang 2007). All PPD individuals were used as the reference population for the background allele frequency calculation. We compared the average relatedness between female dyads and male dyads and tested for significance through 1000 bootstrap re-samplings of the individuals from the observed data set and comparison of the differences in the observed and re-sampled data sets. To show deviations from the population mean, the r estimates were corrected by calibrating the population mean to zero.

In addition, and for comparative purposes, r estimates were also computed with three other estimators: (i) the coefficient of Queller & Goodnight (1989), which is frequently used in the literature and (ii) the coefficients of Wang (2002) and (iii) Lynch & Li (Lynch 1988; Li *et al.* 1993) chosen on the basis of their performance in an estimator evaluation conducted as detailed in the Materials and methods and Supporting information.

Results

Statistical analyses

MtDNA diversity patterns and spatial distribution

We investigated mtDNA diversity and haplotype-sharing patterns. In total, we found 10 different mtDNA haplotypes in Tuanan (Fig. 1; see Supporting information), with an overall, haplotype diversity h of 0.66 (SD ± 0.081) and nucleotide diversity π of 0.006 (SD ± 0.002). Two haplotypes were specific to females: haplotype B was found in four females (10% of individuals) and haplotype C in two females (5%). Another haplotype (A) was very common, found in 23 individuals, and shared by both males (35%) and females (22.5%). The other five haplotypes were all male-specific: haplotype D was present in three males (7.5%), haplotypes E and I in two males each (5%), and haplotypes F, G, H and J in one male each. Interestingly, two of the rare haplotypes unique to the males differed by at least nine mutational steps from the other haplotypes (Fig. 1). The mtDNA variation between the sexes led to a ten-fold higher mtDNA nucleotide diversity in males (π = 0.01 ± 0.002) compared to females (π = 0.001 ± 0.0003). The randomization procedure revealed a significantly higher haplotype diversity in PPD males compared to PPD females (Δ obs ($h_{m/f}$) = 0.102, P = 0.008). Both the presence of sex-specific haplotypes and the significantly higher haplotype diversity in males compared to females are consistent with the genetic predictions for female philopatry and male-biased dispersal.

We were also able to examine the spatial distribution of females, since their home ranges are smaller than those of males and than the study area. While females with haplotypes B and C have their home ranges mainly within the study site, the females with haplotype A range partly in the periphery. Nonetheless, the analyses show

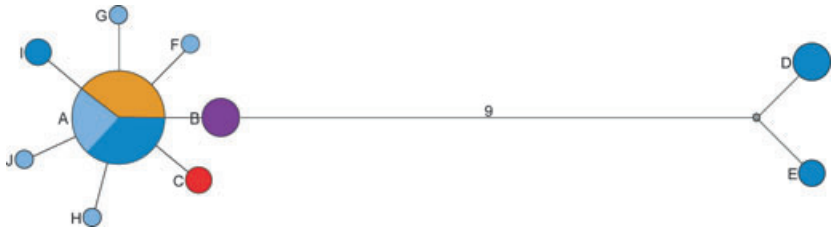

Fig. 1 MtDNA haplotypes in Tuanan. A median joining network of mtDNA haplotypes found in Tuanan is shown. Each different haplotype, shown as a circle, is coloured to represent the proportion of individuals sharing a haplotype: dark blue (flanged males), light blue (unflanged males), other colours (females). Number of mutations between haplotypes is one unless specified.

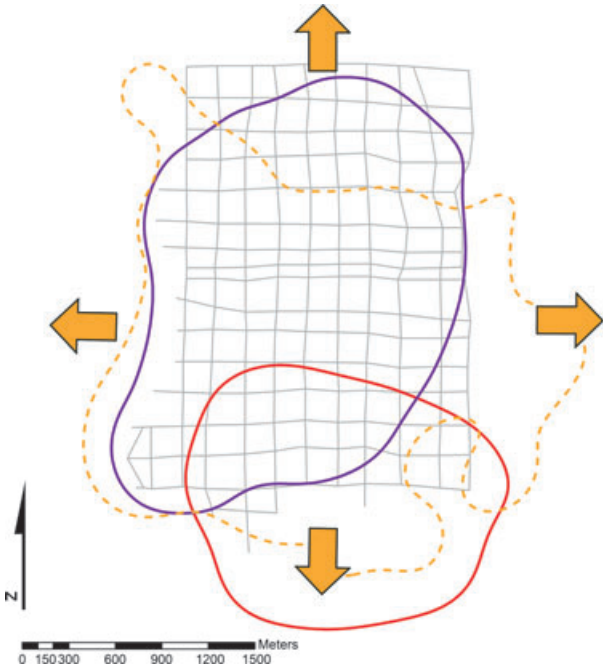

Fig. 2 Spatial distribution of mtDNA lineages in Tuanan. The grid represents the study site, with the combined ranges of females with the same haplotype represented by lines, colour-coded following Fig. 1. The dashed line corresponds to females that frequently moved out of study area (as highlighted by the arrows).

that, within the study site, there is extensive overlap of different mtDNA lineages, indicating that females with different haplotypes share space (Fig. 2).

Parentage-based pedigree analyses

Through the reconstruction of parentage-based pedigrees, we were able to examine the distribution of maternal and paternal relatives among females and males (Table 1). All maternal relationships were confirmed by the observed haplotype sharing. We found that 10 of 15 PPD females had a mother or a PPD daughter at the study site, while only 1 of 24 PPD males was assigned a mother, supporting a model of female philopatry and male-biased dispersal. Particularly the females ranging fully or largely within the study area, those with haplotypes B and C, formed clusters of related individuals. Our results indicate that cluster B comprises a mother and her three adult daughters, two of which in turn have adolescent female offspring. The two PPD females of cluster C were confirmed as a mother–daughter pair. Among the nine PPD females of cluster A, most of which range partly in the periphery of the study site, two mother–daughter pairs were found. The only PPD female with haplotype A and a home range mainly within the study area was not found to have PPD relatives in the area. Field observations indicate that this female had gradually moved from the disturbed habitat in which she had formerly ranged and was consistently chased away at every encounter with other PPD females. None of the males shared haplotypes with the well-known females from clusters B and C, indicating that this is not their natal area. No fathers or paternal relatives were assigned to any of the PPD females or males, indicating that the fathers of adult individuals are not likely to be in the study area.

Relatedness analyses

The average pairwise relatedness estimate *r* as computed with the TrioML estimator was significantly higher among females than males (P value <0.05; Fig. 3). This result was independent of the estimator used, as observed in the comparison across estimators (Fig. S1, Supporting information). We also estimated biparental relatedness for same-sex individuals from the same mtDNA lineage using the TrioML estimator (Fig. 3). The *r* estimates for females with the same mtDNA haplotype were higher than those obtained when all females were pooled together. Males sharing an mtDNA haplotype, by contrast, did not show higher biparental relatedness than all males, irrespective of haplotype. Among individuals with haplotype A, relatedness among females was also significantly higher than that among males.

Discussion

We integrated spatial, observational and genetic data to investigate the dispersal pattern in a nongregarious

Table 1 Maternal and paternal relatives of females and males at Tuanan

		With maternal relatives				With paternal relatives			
Sex	*N*	Mother (%)	Daughter/Son (%)	Sister (%)	Brother (%)	Father (%)	Daughter/Son (%)	Sister (%)	Brother (%)
Females	15	6 (40)	4 (27)	3 (20)	1 (6)	0 (0)	–	0 (0)	0 (0)
Males	24*	1 (4)	–	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*For seven of the males, autosomal genotypes were available for the six loci used in the identity analyses, determined to be powerful for parentage assignments in assessments of marker informativeness (see Supporting Information).

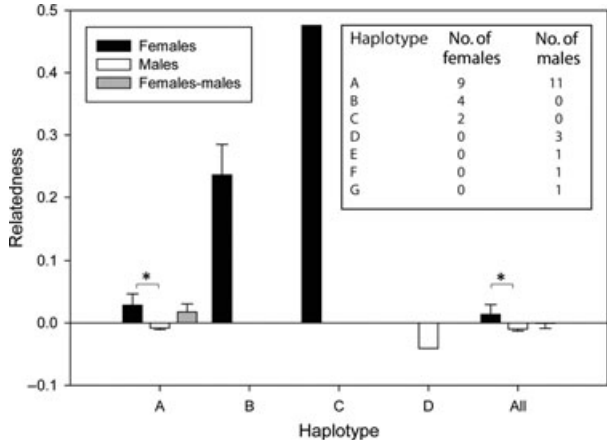

Fig. 3 Female and male biparental relatedness. Trio ML relatedness estimates corrected for population average, as well as variances (error bars) are shown for: all same-sex individuals, and same-sex individuals sharing an mtDNA haplotype. The statistically significant differences in relatedness (P-value < 0.05) are represented by asterisks. For each haplotype, the number of PPD females and PPD males for which complete autosomal genotypes were obtained (microsatellite markers) is detailed in the embedded table.

mammal for which previous genetic studies had produced mixed results. We tested relatedness patterns among individuals through three conventional genetic analyses: mtDNA diversity and haplotype-sharing analyses, the reconstruction of genealogical relationships through parentage analyses and average biparental relatedness. Our results revealed the presence of sex-specific haplotypes and significantly higher mtDNA diversity among males compared to females, underscoring the divergent maternal ancestries of the males. The average pairwise relatedness estimate was higher for females than for males. More importantly, while two-thirds of all females in our study had maternal relatives, with only one case this was the exception for males, indicating a pronounced pattern of female philopatry and male-biased dispersal.

Female philopatry and male-biased dispersal: evidence and comparisons

Among the females, we found three different mtDNA lineages containing clusters of close maternal relatives. For the females with haplotypes B and C, whose home ranges were mainly within the study site, we were able to fully disentangle maternal relationships. Although the relationships among females with haplotype A, most of which range peripherally, are less complete, we did detect two PPD mother–daughter pairs that ranged in the periphery of the study area.

While the female philopatric tendencies supported by our results are congruent with the dispersal patterns in some other solitarily foraging primates, some marked differences are apparent. Notably, there is extensive overlap in home ranges among these females, resulting in spatially stacked matrilineal clusters. These stacked matrilineal clusters contrast with the more spatially distributed maternal lineages in, for example, Coquerel's dwarf lemurs. For this species, Kappeler *et al.* (2002) showed that the sighting centres of females from the same mtDNA lineage are closer than those of females from different lineages. Moreover, within orang-utan clusters, we provided evidence that females are mainly first and second-degree relatives, comprising families of adult mothers and their adult daughters as well as their offspring, while the precise genealogical relationships of females in other nongregarious species are often not known or taken into account, although they may affect nepotistic interactions.

The males in this study, however, differed from the females in several ways. First, they had a far higher diversity of mtDNA haplotypes, most of which were sex-specific. The seven rare haplotypes pertaining exclusively to males highlight their different maternal ancestry compared to the females. This pattern of male-specific haplotypes mirrors the results of studies in the grey mouse lemur and Coquerel's dwarf lemur (Kappeler *et al.* 2002; Wimmer *et al.* 2002; Fredsted *et al.* 2004). Second, males rarely had first-degree relatives in the study area. It was especially revealing that males did not have any mothers or maternal sisters in the study area, except in the case of one young, probably predispersal, male. As none of the males shared maternal ancestry with the well-known centrally located females from clusters B and C, our results indicate that the study site is not a natal area for any of the males. In addition, data on the number of new distinct individuals identified each year indicate that new males keep coming into the study site, while the females are limited in number and well-known after a few years (Fig. S3, Supporting information).

Together, our results match the predictions for a model of female philopatry and male-biased dispersal, in line with previous studies of historical gene flow patterns (Arora *et al.* 2010; Nater *et al.* 2011) and behavioural observation at several orang-utan research sites (Galdikas 1985a; Mitani 1989; van Schaik & van Hooff 1996; Delgado & Van Schaik 2000). Our findings also agree with a recent broad-scale study comparing mitochondrial and Y-linked genetic markers, which provided evidence that orang-utan males move much further than females (Nietlisbach *et al.* in press).

Nevertheless, the patterns we found do not dismiss possible variation in the distances travelled by males, nor a potential range expansion. Some males shared the common haplotype A with the females ranging partly

outside the study area. Thus, it is possible that, unless haplotype A is extremely widespread in the population, these males have their maternal relatives not too far from the study area, suggesting that they have travelled short distances. As male ranges are large and surpass the size of the study site, it is not fully clear whether the males with haplotype A have home ranges that include their natal area, and if so, whether this feature is permanent or temporary, i.e. restricted to early stages of dispersal. Thus, there is a possibility that males with the common haplotype A have expanded their natal ranges, as occurs for instance with bottlenose dolphins (Krützen *et al.* 2004b).

In some cases, males shared their mtDNA haplotype with each other and thus could be maternally related, despite the negative r for males sharing a haplotype as compared to the population mean. Because a parentage-based pedigree reconstruction requires sampling the shared mother to make inferences on shared sibship, inferences on their genealogical relationships cannot be made. However, even if these males were maternally related, parallel male dispersal is unlikely given low male sociality (Delgado & Van Schaik 2000; Utami Atmoko *et al.* 2009). It is nonetheless possible for related males sharing maternal ancestry to converge at a site if the dispersal options are limited because of forest fragmentation and other ecological barriers. This is unlikely to hold for Tuanan, but may be an important consideration elsewhere.

Another interesting finding was that some of the rare sex-specific mtDNA haplotypes were found among unflanged males, who have not yet developed the irreversible secondary sexual characteristics found in the generally older flanged males (Delgado & Van Schaik 2000; Utami *et al.* 2002). Thus, in contrast to suggestions by Morrogh-Bernard *et al.* (2011), our findings indicate that male dispersal may occur when individuals are still young.

Factors affecting the power to disentangle dispersal patterns

Our investigation highlights the importance of several factors affecting the sensitivity of genetic approaches to measure dispersal, particularly for nongregarious species: sampling regime, life history traits and the spatial distribution of individuals.

First, we were better able to resolve the pedigree of females whose home ranges were fully or largely within the study site (cluster B and C), compared to that of females who only partially ranged within it (cluster A). This finding points to the critical importance of size of the sampling area relative to home range size, particularly in nongregarious species. While group-living species have cohesive distinct units of regularly interacting individuals that determine which individuals are sampled, the absence of such units in nongregarious species means that sampling is opportunistic, i.e. spatial rather than group-based criteria, resulting in potential discrepancies between behavioural and genetic results. Especially, the widely used average biparental relatedness estimates are subject to biases stemming from such opportunistic sampling. Species with relatively small home range sizes and small dispersal distances, relative to the sampling area, allow researchers to incorporate larger sample sizes. However, such a sampling regime might lead to the inclusion of unrelated members of the philopatric sex, resulting in lower r estimates than expected. One solution in this case is to measure genetic relatedness against spatial distance (Prugnolle & de Meeus 2002), as has also been performed for various group-living species (i.e. red deer, Nussey *et al.* 2005). To date, studies of a number of nongregarious small-distance travelling mammals show, in agreement with patterns of female philopatry, the expected decrease in female r estimates with increasing geographical distance, and little or no distance effect for males (Coquerel's dwarf lemurs; Kappeler *et al.* 2002; raccoons; Ratnayeke *et al.* 2002; Quail ridge woodrats; McEachern *et al.* 2007). Nevertheless, this approach is not always possible, especially for species with relatively large home ranges and large dispersal distances. Including individuals whose home ranges are not fully encompassed within a study area will reduce the genetic power to detect philopatry if these individuals have their relatives elsewhere.

Second, the slow life histories of some species such as orang-utans and other great apes lead to small sets of closely related individuals at a given time. Thus, in contrast to species with faster life histories, a given sampling area may contain lower numbers of related individuals among the philopatric sex, depending on home range size. It is to be expected then that r estimates decrease with increasing numbers of individuals included in an analysis, as observed in a study of chimpanzees (Lukas *et al.* 2005). Levels of relatedness will also vary depending on reproductive skew, with higher coancestry among the offspring sired by a male with high mating monopolization for instance (Chesser 1991a).

Third, we found stacked matrilineal clusters of females, whose home ranges overlapped. This spatio-genetic structure among females makes it difficult to assess relatedness, as there are both closely related dyads as well as unrelated dyads sharing the same area. This may have been a confounding factor in previous genetic studies of orang-utans, as estimates of average relatedness alone are poor measures of female philopatry. Such spatio-genetic structuring could also

explain cases in other species where, despite behavioural and genetic evidence for female philopatry, average relatedness for females is not higher than expected by chance, as in a study of cougars (Biek *et al.* 2006).

Nonetheless, there may still be some differences in the dispersal patterns across orang-utan populations as a result of intra-specific variation. Such variation would be indicative of facultative dispersal and a high degree of flexibility dependant on population density, local mate and resource competition, and in some cases kin cooperation. In the dusky-footed woodrat, for example, evidence for female kin structures was strongest at intermediate population densities, leading the authors to propose that 'high densities erode kin structures in response to local competition' (McEachern *et al.* 2007). In the grey mouse lemur, despite female philopatry, there is also evidence for the occasional dispersal of females. This was suggested by the spatial conglomeration of females with diverse haplotypes and no obvious female structuring, as well as the presence of multiple clusters of females that were not in spatial proximity but shared the same haplotype (Fredsted *et al.* 2004). In chimpanzees, despite the general pattern of extreme male philopatry and female-biased dispersal, recent research shows great variation in *r* across sites as well as in time (Mitani *et al.* 2002; Nishida *et al.* 2003; Lukas *et al.* 2005). Whereas at sites such as Mahale and Taï, almost all young females emigrate, at Gombe only 50% do and at Bossou none at all. The difference at the latter two sites has been attributed to their lower population sizes and greater isolation from other sites (Mitani *et al.* 2002; Nishida *et al.* 2003). In gorillas, males can either remain in the natal group or leave, and the fitness consequences of dispersal decisions for males at least have been shown to depend partly on demographic variables (Robbins & Robbins 2005). Another interesting possibility is that the recent availability of suitable unsettled habitat, as sometimes accompanies spatial expansions, could change the benefits and costs of dispersal, for instance, by increasing the fitness of dispersers.

Matrilineally related kin structures in orang-utans might confer social benefits to females. Despite the low levels of sociality displayed by orang-utans, associations do occur and have been shown to be more likely among related than unrelated females (van Noordwijk *et al.* 2012). Such associations provide opportunities for play among the offspring of closely related females (van Noordwijk *et al.* 2012). Taken together, these findings support suggestions by Singleton & van Schaik (2002) for the role of nepotistic tolerance in determining the nature of social interactions and opportunities to acquire new skills. Nepotistic tolerance might also make settlement in overlapping home ranges easier for relatives than nonrelatives. Given these results, kin selection may be an important evolutionary mechanism underpinning matrilineal kin structures not only in orang-utans but also in other nongregarious species where these structures remain underexplored, and which warrant detailed investigation.

Acknowledgements

We thank Jinliang Wang for his comments on the manuscript. We are also grateful to Pascal Marty and Claude Rosselet for their help with GIS work. This research would not have been possible without the help of the Indonesian Institute of Sciences and the Indonesian State Ministry for Research and Technology for granting permission to undertake this research. We also thank the A.H. Schultz foundation for additional funding. We are especially indebted to all assistants and students who helped collect and coordinate samples and behavioural data in the field. Samples were exported from Indonesia to Switzerland under permits 07279/IV/SATS-LN/2009, 00961/IV/SATS-LN/2007 from the Convention on International Trade in Endangered Species of Wild Fauna and Flora.

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N.A., C.A., A.N., M.G., P.N., and M.K. apply genetic methods to study evolutionary, population genetic and ecological questions in wild animal populations. M.K. and E.P.W. investigate the evolution of culture and cooperation using genetic and spatial data. S.S.U.A., J.P., and D.P.W. study Indonesian primates. L.D., Mv.N. and Cv.S. are interested in the social evolution and cognition of primates.

Data accessibility

The mtDNA HVRI sequences have been deposited in EMBL under accession numbers FR717918–FR717919 and FR717921–FR717924. The genotypes and haplotypes for each individual, as well as the spatial distribution data for the females, are provided in the Supporting information.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Supporting Information Methods and Results.

Appendix S2 Supporting Information Tables and Figures.

Appendix S3 Supporting Information Genotypes and Haplotypes.

Appendix S4 Supporting Information Haplotypes.

Appendix S5 Supporting Information nexus file Tuanan New Haplotypes.

Appendix S6 Supporting Information Ranging Data.

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