

A 50 year decennial survey of male Bornean orangutans (*Pongo pygmaeus wurmbii*) with supplemental genetic analysis in Tanjung Puting National Park, Indonesia

BIRUTÉ MARY GALDIKAS^{1,2}, RUTH ELLA LINSKY^{1,3,*}, RENIASTOETIE DJOJOASMORO², JOSEPH LORENZ⁴, R. STEVEN WAGNER⁵

¹Department of Biological Sciences, Simon Fraser University. 8888 University Drive, Burnaby, British Columbia, Canada v5A 1S6. Tel.: +1-778-782-4475, *email: ruth_linsky@sfu.ca

²Orangutan Foundation International. Jl. Tebet Barat Dalam VI A No. 9, South Jakarta 12810, Special Capital District of Jakarta, Indonesia

³Primate Behavior Program, Central Washington University. 400 E University Way, Ellensburg, Washington 98926, USA

⁴Eichendorffstraße 32, 68167 Mannheim, Germany

⁵Biotecon Diagnostics, Hermannswerder 17, Potsdam 14473, Germany

Manuscript received: 12 January 2023. Revision accepted: 10 May 2023.

Abstract. Galdikas BM, Linsky RE, Djojoasmoro R, Lorenz J, Wagner RS. 2023. A 50 year decennial survey of male Bornean orangutans (*Pongo pygmaeus wurmbii*) with supplemental genetic analysis in Tanjung Puting National Park, Indonesia. *Biodiversitas* 24: 2587-2595. This study investigates the declining presence of adult males in a protected wild population of Bornean orangutans (*Pongo pygmaeus wurmbii*) at the Camp Leakey study area in Tanjung Puting National Park (TPNP), Central Kalimantan, Indonesian Borneo. Orangutan populations are in decline due to habitat destruction and fragmentation. Documenting changes within existing orangutan populations over time is important to understand demographic changes that may indicate pre-extinction processes and imminent collapse. Wild orangutan behavior has been studied at Camp Leakey since 1971. Observations of orangutans over the past five decades have documented a greatly decreased number of encounters with flanged adult males. A supplementary six-month genetic study was hindered by the fact that only two adult males briefly appeared in the study area in 2016 as compared to 40 adult male encounters in the same time period in 1976. The decline which appears most visible in data from 1986 to 1996 continues into 2006 and 2016. Genotypes from 24 wild adult orangutans encountered in 2016 were analyzed to assess the expected genetic parameters of a wild orangutan population. Pairwise relatedness was high among and between adult females and subadult males. The only two flanged males present during the genetic survey shared zero pairwise relatednesses. However, both flanged males had non-zero pairwise relatedness with other members of the Camp Leakey community and thus it is likely they were from different local areas. These data show the genetic consequences of the observed demographic changes. As TPNP forests have become disconnected from neighboring habitats, natural orangutan male dispersal may be disrupted. Disruption of male dispersal would increase the risk of accelerated extirpation for the orangutan population in TPNP as well as other orangutan populations, especially those that are remnant and less protected.

Keywords: Dispersal, extinction, genetic diversity, habitat fragmentation, relatedness, SNP

Abbreviations: CL: Camp Leakey; OFI: Orangutan Foundation International; TPNP: Tanjung Puting National Park

INTRODUCTION

As wild primate populations shrink and habitats are increasingly disturbed by human activity, intact primate population parameters and dynamics need to be documented so that evolutionary processes and ecological adaptations leading to extinction can be better understood. This is especially the case for great apes, including orangutans (*Pongo* spp.) who are unique in being specially adapted to live semi-solitary arboreal lives in dense primary rainforest. These unique ecological and social adaptations result in diffuse and widespread populations which are difficult to define. Nevertheless, it has been known that orangutan populations across their range have been in decline for decades (Utami-Atmoko et al. 2017). This has different effects over time on different age-sex classes of orangutans. As populations become small, demographics may change and the negative effects of loss of genetic variation through

genetic drift and inbreeding can increase (Wright 1931).

The Camp Leakey study area is located in northern Tanjung Puting National Park (TPNP), Central Kalimantan, Indonesian Borneo. The wild orangutans within the study area have been the focus of ecological and behavioral studies spanning the last five decades (Galdikas 1979, 1982, 1983, 1985a, 1985b, 1985c, 1988; Galdikas and Wood 1990; Hamilton and Galdikas 1994; Muir et al. 2000; Banes et al. 2016; Linsky et al. 2022). Individuals within the study area represent one of the two largest remaining wild populations in Borneo and thus should represent naturally evolved Bornean orangutan (*Pongo pygmaeus*) behaviors, ecology, and genetics that have already likely been much disturbed in most other areas where only fragmented forests remain. This population is also unique as it has been behaviorally observed through periodic searches and focal following for the past five decades. The northern area and orangutans of Tanjung

Puting were relatively untouched before Galdikas' arrival in 1971. The northern forests have benefitted from protection due to her establishment of Camp Leakey and research and conservation programs in the area, as well as the Indonesian Government's important declaration of Tanjung Puting's change in status from Wildlife Reserve to National Park in 1982. Adding to its uniqueness, this large orangutan population is geographically partially confined as TPNP is situated on a peninsula. TPNP consists of approx. 411,000 ha jutting out south into the Java Sea. The peninsula, larger than TPNP alone, is naturally isolated from the west and south by the Java Sea and by the lower reaches of the Sekonyer River, and from the east by the Seruyan River which throughout much of its length has a width equivalent to the Mississippi. Connectivity with the wider historical orangutan populations probably would have been only possible from the north of the peninsula. Even though the southern part of the park has a recent history of fires and illegal logging, the northern part has been largely undisturbed for at least the last 50 years (Galdikas and Shapiro 1994; Brend 2006). However, the primary forest of the peninsula has, over the last two decades, become nearly totally separated from the northern mainland forest habitat due to the conversion of large swaths of forest to oil palm plantations and human economic development. Although the total orangutan population within Tanjung Puting has been estimated from between ~4,000 to ~12,000 (Galdikas et al. 2004 unpublished data; Utami-Atmoko et al. 2017), orangutans attempting to disperse from or into this area may now be seriously impeded by areas lacking wild food resources and high risk of human conflict found in surrounding large scale industrial oil palm estates. The emerging and potential consequences of this increased isolation of an already partially geographically isolated wild orangutan population have yet to be documented or described.

Orangutans have been documented through behavioral studies at Camp Leakey and at other sites by observational and genetic studies to be female philopatric (Galdikas 1979; Goossens et al. 2006; Arora et al. 2012; van Noordwijk et al. 2012) and to live in a matrilineal network of females and local unflanged males. Subadult males disperse from their mother's natal range after achieving foraging independence and often become nomadic. Flanged adult male orangutans sometimes range widely but at times establish periods of semi-permanent residence in smaller areas. Males, both mature males with facial flanges and unflanged subadult males, may disperse long distances (Galdikas 1985a, 1985b, 1988; Nater et al. 2011; Nietlisbach et al. 2012). However, illuminating the genetic signature of male-biased dispersal within populations of orangutans long term has been slow due to difficulties accessing and sampling these diffuse and cryptic apes. There have been several published studies specifically on relatedness and some other metrics of genetic diversity within populations in Borneo and one from a study site in Sumatra (Utami et al. 2002; Goossens et al. 2005, 2006; Morrogh-Bernard et al. 2011; Arora et al. 2012). Results have varied at each of these sites with some studies finding higher relatedness amongst adult females than males and some finding more equal relatedness within both sexes. The

majority of studies have not found high pairwise relatedness among flanged males except for one study conducted in Kinabatangan, northern Borneo (Goossens et al. 2006) where orangutans seem predominantly crowded to river edge habitats.

Our study seeks to add to the mosaic of data for wild orangutan demographic parameters and to better understand the dynamics occurring within the Camp Leakey study population. We establish that a resounding change in flanged adult male presence occurred over 50 years. Using preliminary data from a 6-month genetic survey in 2016 we have tested for and identified possible genetic signals of this change (Linsky et al. 2022). We hypothesized that the Camp Leakey area should exhibit genetic diversity and relatedness, which we find in other wild orangutan populations according to what is known of female philopatric behavior and male-biased dispersal patterns. To test this hypothesis, we investigated three distinct genetic expectations.

First, relatedness among female philopatric lineages is expected to be higher than relatedness among adult males who are the dispersing sex. Adult (flanged) males with cheek pads who move into the area should be unrelated or less related (or not related) to females when compared to others in the area. Relatedness among subadult unflanged males who have not yet dispersed from their natal ranges may be predictably higher than that of immigrant flanged males. Second, Chesser (1991) showed that genetic diversity among philopatric female groups in species exhibiting polygyny also tends towards excess heterozygosity (Het Excess), or negative inbreeding coefficient (F_{is}) estimates within the lineages. This excess heterozygosity is dependent on the number of breeding males. The more breeding males, the more heterozygosity is kept up within the philopatric groups. As orangutans are known to be polygamous, if there are sufficient breeding males in the area and if we have sampled females from philopatric matriline lineages, females should show higher Het Excess and more negative F_{is} , than among the total adult population sampled. Third, if males are immigrating from afar, adult males may have a higher observed heterozygosity and average allelic richness than the local philopatric females as well as more unique or rare alleles. We proposed that deviations from these expectations may show early genetic signs of disrupted natural orangutan dispersal patterns and signals of much faster demographic consequences of population isolation indicated by the decreased adult male presence within the study area. These expectations are tested using two genotype sets. One set of autosomal microsatellite (Short Tandem Repeat, STR) markers were used as well as a set of homologous human derived Single Nucleotide Polymorphisms (SNPs) uniquely discovered in this sample of wild orangutans at the Camp Leakey study area through cross-species genotyping on a SNP chip designed for human use published in Linsky et al. (2022).

MATERIALS AND METHODS

Study area

The Camp Leakey study site is located at 2°45'-48'S latitude and 111°57'-112° 1'W longitude within the

Tanjung Puting National Park in Central Kalimantan, Indonesian Borneo. The National Park is approximately 4,110 km² in size and includes a mix of dry ground tropical heath and dipterocarp forests with areas of permanently wet and seasonally flooded peat swamps throughout (Galdikas 1979). The CL study area is a 35km² area of regularly maintained trails throughout which local habituated and transient wild orangutans have been behaviorally studied through focal follows since the early 1970s. Although the “formal” study area is 35 km², research staff occasionally follow focal individuals outside this area. The study area includes difficult terrain such as deep swamps and semi-vertical short slopes.

Procedures

Orangutan encounter data

Orangutans are difficult to locate in primary dry ground and peat swamp forests. They are primarily solitary, silent, and spend much time sitting and resting immobile in the canopy. In order to periodically observe orangutans, it is necessary to first search for them. Over 125 kilometers of cut, trails were made in the Camp Leakey study area to facilitate this search process. Trails are laid out approximately 500m apart North-South and East-West and tagged every 25m. Galdikas and pairs of researchers use the trail system to search for and locate wild Bornean orangutans and carry out intensive focal following behavioral data collection. Successful orangutan searches took up to 20 days of constant dawn-to-dusk trail-walking. As orangutans are largely tree dwelling, researchers must search the canopy for orangutans and observe the behavior from the ground using direct observation. Researchers move along the ground keeping a distance from the orangutan so as not to disturb natural behaviors. Each searcher uses a pair of binoculars to identify and observe orangutan behavior. Search data are recorded in terms of trails traversed, time when the orangutan is first located and the behavior of the orangutan recorded when located. Recorded follow data consist of bouts of activity, the height of orangutan in the canopy or presence on the ground, habitat type, depth of water in the swamp, type of food and

species being eaten, and travel direction, with maps being drawn assisted by compass and trail markers. Behaviors are recorded from the first encounter with the orangutan until evening when they make a night nest in the canopy for sleeping. Researchers note the location and return to camp. Before dawn the next day, researchers return to the nest location via trails and await the first movement of the orangutan for the day. The remaining following days are from morning nest to night nest. Any encounters with other orangutans during a focal follow are recorded and each additional orangutan is identified when possible. Orangutans are generally followed for a duration of 10 days at which point they are left and another orangutan is located. Occasionally a located focal orangutan was followed for longer than 10 days, up to 65 days. However, this was exceptional. The same search and follow procedures have been conducted since 1971 by Galdikas, long-term staff, and Indonesian students. The number of simultaneous searchers did change over the five decades sampled. Before 1976 Galdikas and Rod Brindamour were the two main investigators, with one local assistant and then two Indonesian students who joined in 1974. This meant that up to five people could search and follow at one time. By 1986 the experienced local staff had increased in number and up to 8-10 local staff were in the field searching and following simultaneously at Camp Leakey. In 1996 the number of searches and followers returned to numbers similar to 1976 and have remained relatively consistent up until the present day.

Wild orangutan observational data from wild orangutan focal follows was collated for the years 1976, 1986, 1996, 2006, and 2016. The number of unique adult and subadult males encountered within the study area was counted and totaled for each month, and then summed over the time period from February-August of each of those years as this coincides with the same six-month period as the genetic survey conducted in 2016. The search effort was collated by counting the number of individuals (if following or searching alone) or groups (2-4 people) following or searching each day over the month.

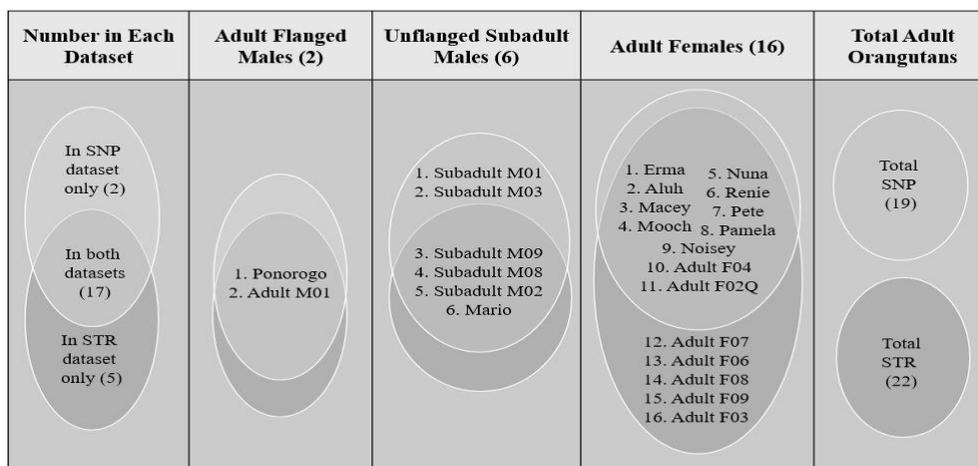


Figure 1. Flanged adult, unflanged subadult male, and adult female orangutans in each dataset. Those with SNP genotypes are in the lighter circle on top and those with STR genotypes are in the darker circle below. Those individuals genotyped with both methods are in the combined space

Genetic sampling, genotyping and analysis

Between February and August of 2016 fecal samples were collected passively from beneath nests or from the ground as observed once dropped from wild orangutans within the CL study area (except two adult female samples collected once in 2013 and once in 2014). Individuals were located and followed using the standard focal follow procedure described. We obtained and assigned multiple fecal samples per individual as well as identification photos of each orangutan sampled.

Samples were collected and stored as per a two-step method (Nsubuga et al. 2004) described in Linsky et al. (2022). Samples were stored frozen until exported from Indonesia under the CITES export permit 01152/IV/SATS-LN/2017. DNA extraction and genotyping of two types (STR and SNP) were conducted as outlined in Linsky et al. 2022. SNP genotyping resulted in the identification and assignment of 125 homologous autosomal human-orangutan SNPs for 19 unique adult or independent individual orangutans (Linsky et al. 2022). Genotypes for eight autosomal tetra-nucleotide microsatellite STR loci, consisting of five *Pongo*-specific (Nietlisbach et al. 2010) and three human-specific (Goossens et al. 2005), were produced for 22 unique adult individuals. Sixteen of the individuals were genotyped by both methods, two additional males were SNP genotyped and five additional adult females were STR genotyped, resulting in a total of 23 unique individual orangutans. The breakdown of individuals for each dataset is found in Figure 1. All adults, flanged and unflanged males and females were found to range independently and considered to be autonomous adults. Details about each individual genotyped and any known (observationally recorded) relatedness between them can be found in Linsky et al. 2022. Relatedness values for all individual pairs calculated from STR and SNP datasets were correlated as reported by Linsky et al. (2022). Genotypes created were subsequently used here to estimate relatedness and diversity between and within sex-age classes.

Allele frequencies were calculated and distinct individual identities for both the SNP and STR datasets were confirmed using Cervus (Kalinowski et al. 2007). Pairwise relatedness (r) values were calculated using the triadic likelihood estimator, TrioML (Wang 2007) by the Coancestry (Wang 2011) software. To compare relatedness between groups, a bootstrapping method was used as per the Coancestry software. Dyads from each group were drawn randomly 1000 times to produce a distribution of the average relatedness for each group and the difference between groups. The observed difference was then compared to this distribution for significance at the 95 percentiles.

Estimates of inbreeding coefficient (F_{is}), mean number of STR genotypes (N), number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), and unbiased expected heterozygosity (uH_e) for each age-sex group were calculated with the GENEPOP online web program (Raymond and Rousset 1995; Rousset 2008). Heterozygosity excess (Het Excess) at each SNP locus was output from the GenomeStudio2.0 genotype calling software (Illumina, Inc.). Two-tailed T-tests for statistical

differences between means were used to compare differences between sex class groups.

RESULTS AND DISCUSSION

Results

The total monthly male orangutan encounters within the Camp Leakey study area decreased considerably from 1976 to 2016. Five years into her study, in 1976, Galdikas and four other data collectors encountered adult and subadult males 40 times, of which 25 were flanged males, in 170 search/follow days over the six-month period of February-August of 1976. This was the largest number of males encountered in the time periods sampled. The total number of monthly males encountered decreased to 35, of which 21 were flanged males, in Feb-Aug of 1986 despite a much higher number of search/follow days, 507, and searchers in that time period. The number of male orangutans encountered dropped off precipitously from 1986 to 1996 (15 males, of which 11 were flanged) and then 2006 (5 males, of which 2 were flanged) as the number of search/follow days returned to numbers similar to 1976, 242 days and 177 days respectively. The total number of males increased slightly in 2016 (8 males) of which only two were flanged males. Figure 2 shows the number of encounters of adult flanged and subadult males for the years 1976, 1986, 1996, 2006, and 2016 and the total search and follow effort for each time period.

Overall TrioML maximum likelihood adult relatedness in the 19 adults (2 flanged males, 6 unflanged subadult males, and 11 females) in the SNP dataset was 0.088 (var = 0.018). Relatedness between the two flanged adult males was 0.000. The average relatedness among the subadult males was 0.111 (var = 0.038) and the average adult female relatedness was 0.106 (var = 0.019).

Bootstrapping tests found female SNP relatedness and average subadult male SNP relatedness were significantly higher than the relatedness between the two flanged males ($r = 0.00$), who were unrelated. SNP relatedness between subadult males and females ($r = 0.092$) was not significantly different than SNP relatedness between flanged males and females ($r = 0.058$). Each flanged male average SNP relatedness to females was significantly higher than 0.

Overall TrioML maximum likelihood adult relatedness in the 22 adults (2 flanged adult males, 4 unflanged males, and 16 females) in the microsatellite STR dataset was 0.073 (var = 0.018). STR relatedness between the two flanged adult males was also 0.00. Average unflanged male relatedness was 0.187 (var = 0.001) and average adult female relatedness was 0.077 (var = 0.021).

Bootstrapping tests also found that average female STR relatedness and average unflanged male STR relatedness were significantly higher than 0.00 (flanged male STR relatedness). Bootstrapping tests found mean unflanged male STR relatedness to females was significantly higher than the mean flanged male STR relatedness to females. Mean flanged-male STR relatedness to females was significantly higher than 0.

Relatedness values between flanged and unflanged males and adult females for both SNP and STR datasets are shown in Table 1. Average SNP Het Excess across all 19

adults and all 125 SNP loci was 0.016. SNP Het excess across the 11 adult females was 0.106. Average STR inbreeding coefficient estimates (Weir and Cockerham's (1984) F_{IS} across all 22 adults was 0.029. Average adult female (W and C) F_{IS} was -0.015. Averages estimated with Robertson and Hill's (1984) F_{IS} estimator across all 22 adults was 0.545 and across the 16 females was -0.098.

Unbiased expected heterozygosity (H_e) for all 22 adults across the 8 STR microsatellite loci was 0.66 (var = 0.012) and observed heterozygosity (H_o) was 0.64 (var = 0.020). Mean number of effective alleles across all loci and adults was 3.04 (SE = 0.027). Values for mean number of alleles, effective number of alleles, observed and expected heterozygosity for each group of adult flanged males, adult non-flanged males, and adult females using the STR genotypes are listed in Table 2.

Two-tailed t-tests for uneven variance showed adult flanged male STR H_o across the eight loci to be not significantly different from adult female STR H_o ; $t(9) = -0.902$, $p = 0.390$. Interestingly, adult females had significantly more alleles and effective alleles across the 8 loci than flanged males; $t(10) = -3.930$, $p = 0.003$ and $t(11) = -2.476$, $p = 0.031$.

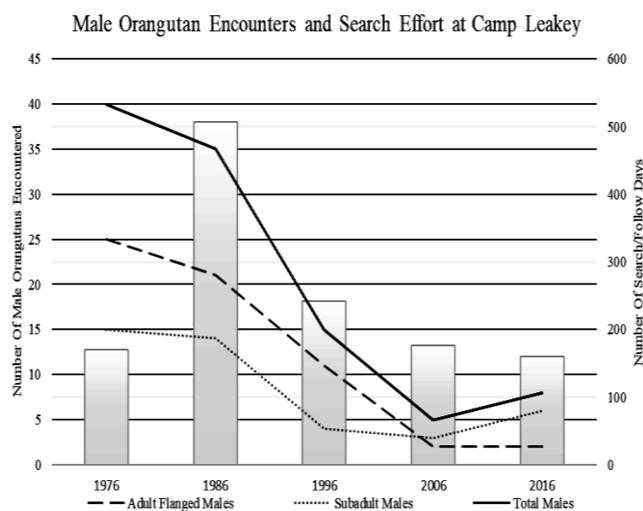


Figure 2. Number of monthly male orangutan encounters totaled six months (February-August) in a decennial survey across 50 years. A solid black line represents the total number of males encountered, the total number of adult males is represented by a dashed line, total subadult males by a dotted line. The total number of search and follow days for each time period is shown with grey bars on the second (right) y-axis

Table 1. Average pairwise relatedness between age-sex groups with values from each genotype dataset noted as SNP (STR)

	Adult flanged males	Subadult male	Adult females
Adult flanged males	0.00 (0.00)	-	-
Subadult males	0.080 (0.054)	0.111 (0.187)	-
Adult females	0.058 (0.034)	0.092 (0.077)	0.106 (0.077)

Table 2. Mean number of STR genotypes N , number of alleles N_a , effective number of alleles N_e , observed heterozygosity H_o , and unbiased expected heterozygosity uH_e for each age sex group

Sex		N	N_a	N_e	H_o	uH_e
Female	Mean	13.625	4.250	2.925	0.656	0.648
	SE	0.565	0.491	0.325	0.057	0.048
Non-Flanged Males	Mean	3.625	2.750	2.226	0.667	0.604
	SE	0.183	0.313	0.212	0.129	0.059
Flanged Males	Mean	1.750	2.125	1.992	0.500	0.646
	SE	0.164	0.227	0.191	0.164	0.107

Discussion

The study population at Camp Leakey is unique in many ways especially in its relative lack of perturbation in comparison to many other forests in Kalimantan (Galdikas and Shapiro 1994; Brend 2006). It has been continuously studied for over 50 years by Galdikas, Indonesian students, and local staff. The orangutan population in the northern part of the park is relatively undisturbed (in comparison to those in surrounding areas).

The paucity of males in the six-month genetic study should be emphasized. The sample is regrettably small because during the intensive six-month genetic sample collection period, only two wild-flanged males were encountered and thus, only two could be sampled. It is unlikely that this is due to sampling error for several reasons. Adult flanged males are the easiest orangutans of all age-sex classes to recognize and identify. Usually, each male is distinctive due to size, shape, and markings of and on cheek pads, as well as hair patterns on the body, beard appearance and length, and missing, stiff, or gibbled fingers and/or toes. Sometimes males have missing or injured eyes and occasionally they have “dreadful” wounds and scars on their faces and upper lips. Young adult males are quite different in appearance from older or elderly ones whose cheek pads seemingly shrink and who may display visible injuries such as stiff legs. Orangutan search and follow effort within the Camp Leakey study area in 2016 was similar to that in 1976 however, very few long calls, which are the vocalizations of flanged males, were heard during search days in the six-month sample collection period of 2016. This low number of flanged males in 2006 and 2016 is clearly unusual compared to historical encounter data from the area (data not shown) when sometimes a number of transient males entered and left the study area in a few weeks. In the first four years of her study (1971-1975) Galdikas documented and named 12 habituated adult orangutan males and nine subadult males (plus a number of unhabituated transient males) within the study area with none of the adult males staying within the study area for the entire four-year period (Galdikas 1988). The subadult males did not seem to stay either but they were encountered at unpredictable intervals (Galdikas 1985c). During the 1976 period, the number of males recorded increased but as Galdikas and local staff grew more experienced in searching for wild orangutans over the next decade, the number of males encountered dropped. Habituated males seemingly “disappeared” while the number of habituated resident females remained relatively constant. In one case, a habituated adult male who had

“disappeared”, briefly reappeared in the study area seven years later and then was never seen again (Galdikas 1995).

Despite the relatively undisturbed history of this population, our genetic data from 2016 only supports two of the three expectations which arise from our hypothesis that the Camp Leakey population should exhibit genetic relatedness and diversity signatures of known patterns of orangutan female philopatry and male dispersal. Pairwise relatedness estimates calculated from both datasets support our first expectation and match values expected in a female philopatric community. With both datasets, average pairwise relatedness among the 16 wild female orangutans and among the six wild unflanged subadult male orangutans sampled at Camp Leakey was high, within the range of half cousins to full cousins (0.065-0.125). A female with a third offspring, estimated three years of age, was assigned to be the mother of two independent males, one a subadult and the younger one an adolescent (Linsky et al. 2022). With the same mother this would make them half-brothers. Average birth intervals in this Bornean population are 7.8 years (Galdikas and Wood 1990) suggesting that the older brother is at least 18 years of age. In the past, he would have left his mother's home range by the time of sampling and probably would have dispersed from the Camp Leakey area (Galdikas 1988c).

Relatedness within the female and subadult male groups was significantly higher than relatedness between the two adult flanged males sampled (whose pairwise relatedness was estimated to be 0.00). Pairwise relatedness between the unflanged males and the adult female orangutans was higher (i.e., significantly higher in the STR dataset but not significantly in the SNP dataset) than pairwise relatedness between flanged males and females, which was significantly, and interestingly, not zero. It seems the six unflanged males sampled were local males from the area who had yet to disperse from near their mothers' home ranges. This also suggests the two flanged males, while not related to each other, dispersed into the Camp Leakey area from nearby, possibly from a neighboring forest, each with extended family members related to the females sampled in this study. The two flanged males may also be potentially paternally related to local females through other males who may also have dispersed into the area and bred with females in the past.

Our second expectation for philopatric females was also supported as adult female orangutans were found in both datasets to have higher heterozygosity excess and lower inbreeding coefficient when compared to the entire adult sample. These results support the hypothesis that we have sampled adult females from several closely related female lineages. These lineages, although closely related, are not suffering from loss of heterozygosity, likely due to the historical immigration of a sufficient number of adult males into the area and mating with these wild female groups, as predicted by Chesson (1991).

Measures of genetic diversity calculated in our sample with both datasets only partially support our third expectation for the signature of distantly immigrant males within our sample set. Observed heterozygosity within the

two flanged males was not significantly different from that of the 11 or 16 adult females (in either dataset). Females had, on average, one more effective STR allele per loci than the two flanged males, and flanged male SNP MAF was not significantly lower than among females, meaning they did not, on average, have more rare alleles. These findings together with our pairwise relatedness results, indicate these sampled flanged males were not from a substantially different population than the local CL community and are likely from the Tanjung Puting peninsula. Although the flanged males were less related to females in the area as compared to the unflanged males, they were not completely unrelated. The lack of males completely unrelated to any adult females in the sample suggests that distinctly new males from outside the Tanjung Puting area were not present during sampling.

Overall higher pairwise relatedness values within adult females than within flanged males in our sample are similar when compared (Table 3) to population values published at two other sites in Central Kalimantan; Sebangau (Morrogh-Bernard et al. 2011) and Tuanan (Arora et al. 2012). Both the Tuanan (7.5km²) and Sebangau (9.0km²) orangutan research sites are within now protected large forests, the Mawas Conservation area (3090 km²), and Sebangau National Park (5140 km²) respectively. These two areas along with Tanjung Puting represent three of the larger remaining orangutan populations within Kalimantan.

The Mawas conservation area can be classified as a lowland peat swamp forest of primary orangutan habitat, despite local illegal logging. However, the swamp in the Mawas Conservation area was once drained through extensive canalling under the abandoned Mega Rice program of the federal government in the 1990s. The Sebangau research site is also within a large contiguous mixed peat swamp habitat, but the forest in this area was selectively logged for more than three decades beginning in the 1970s and also cut by canals draining the peat for the proposed Mega Rice agriculture project. Both of these areas are bounded by large rivers that run from the mountainous ridge of central Borneo south towards the Java Sea.

These river boundaries prevent orangutan dispersal laterally across these coastal forests, thus creating effective peninsulas bounded by these huge waterways. In order for orangutans to traverse, they must move inland first before crossing and returning outward again to coastal areas. These river-bounded peninsulas, both geographic and effective, would provide an explanation for high relatedness within females seeking to stay near their natal ranges but confined to these forest corridors. Thus, this high female relatedness reported across the three sites in Central Kalimantan may be a natural consequence of the geography of the Island of Borneo. As both the Tuanan and Sebangau studies reported unrelated males, it seems males are still able to make movements inland and out again in those areas. However, our results suggest this may no longer be the case in Tanjung Puting. This may be because the areas between respective bounding rivers are not as wide for the Tanjung Puting peninsula.

Table 3. Average pairwise relatedness within and between adult males and females was reported from each site. + positive or high relatedness, - negative or not related

Area	Relatedness within females	Relatedness within males	Relatedness between males and females
Ketambe, Sumatra ^c	-	-	+ ^a between assigned maternities and paternities
Kinabatangan, Sabah Malaysia ^d	+	+	+
Tuanan, Mawas Central Kalimantan ^e	+	-	-
Sebangau, Central Kalimantan ^f	+	-	- ^b did not share any haplotypes
Tanjung Puting	+	-	+

Note: ^atotal average relatedness between all adult males and females was not reported, rather they reported an average relatedness between all adults assigned maternities and paternities, ^brelatedness between males and females was not reported however males did not share any mtDNA haplotypes with females, ^c(Utami et al. 2002), ^d(Goossens et al. 2006), ^e(Arora et al. 2012), ^f(Morrogh-Bernard et al. 2011)

The average adult observed heterozygosity (H_o) across the 125 SNP loci was 0.340. The average adult flanged male ($n = 2$) SNP H_o was 0.300 with an average MAF of 0.160. The average subadult male ($n = 4$) H_o was 0.430 with an average MAF of 0.290. The average adult female ($n = 11$) SNP H_o was 0.310 with an average MAF of 0.190. Two-tailed T-tests for statistical difference between means showed no significant difference between flanged adult male SNP H_o and that of adult female H_o ($t(202) = 0.354$, $p = 0.724$). The male average MAF (0.160) was lower but not significantly lower than the female average MAF (0.190) ($t(231) = 1.613$, $p = 0.108$).

Alternatively, a study conducted in the northern province of Sabah, Malaysia, also on the island of Borneo, reported similarly high relatedness values within males and females, further indicating both sexes as equally dispersing and also equally remaining in natal areas (Goossens et al. 2006). They also found unexpectedly high diversity within the entire population. This study site in the Kinabatangan Conservation Area is comprised of fragmented riparian forest blocks on either side of the Kinabatangan River, creating a ~270km² corridor of habitat along the river that is surrounded by large-scale oil palm plantations. The authors have suggested orangutans in this refuge currently live in “communities of related individuals of both sexes” (Goossens et al. 2006 p.2585) but indicate that this could be the result of intense habitat fragmentation and destruction that occurred largely before the mid-1990s, which may have reduced orangutan dispersal abilities.

A study reporting relatedness within a Sumatran population at the Ketambe Research Station (4.5km²) within Gunung Leuser National Park in 2002 also found similar, but in this case, negative relatedness within males and females at the site. This study population is also situated within undisturbed primary lowland habitat bound on two sides by large rivers. However, this genetic study included ex-captive orangutans who had been introduced into the area, and subsequent offspring. Thus, their finding that individuals were largely not related is not surprising and may not reflect the case within the greater local wild population.

Overall sample wide average heterozygosity within our microsatellite STR dataset of 0.664 falls within average heterozygosity for mammals and primate groups (Garner et al. 2005). However, a very low average number of alleles

($N_a = 3$) for all STR loci was found for both males and females once the sample size difference was taken into account. This N_a is both low compared to other published results for orangutans in Central Kalimantan and other parts of Borneo (Goossens et al. 2005; Nietlisbach et al. 2010; Morrogh-Bernard et al. 2011) as well as with that expected for mammals at large (Garner et al. 2005). The low number of alleles in males may suggest demographic challenges and could indicate that the immigration of new alleles into the TNTP is diminished. We suggest that these findings may be an early indicator of the effects of disrupted male dispersal in the Camp Leakey population. Obviously, a larger sample of males for future genetic studies is warranted.

The Tanjung Puting peninsula is isolated from remaining forests by two rivers and the Java Sea, as well as extensive industrial-scale oil palm plantations to the north. This geography requires that the dispersal of orangutans into the TPNP population must be from the more inland northerly areas, which are now heavily populated and being developed. Dispersal by orangutans from the unprotected forest in this area is perilous, with human-orangutan conflict frequent (Orangutan Foundation International, www.orangutan.org). Our observational data from the Camp Leakey study area indicate that males are not dispersing into or out of this area as they formerly did. Male orangutan encounters at Camp Leakey in 1976 was high. Encountering many male orangutans within the study area was standard throughout that decade and into the next (1980s). Some males were given names and considered residents and were sighted repeatedly throughout a year and even across years but none are still present. Many males were sighted only a few times and then never again, clearly indicating that males passed through the Camp Leakey study area regularly without attaining residence. Male encounter numbers were lower in 1986 despite the fact that search efforts increased as field staff were consistently in the Camp Leakey study area. Drastically lower numbers of males encountered, with roughly consistent search efforts in 1996, 2006, and 2016 (and even up to 2022, Galdikas in preparation), indicates the number of males entering the study area has decreased in the past 30 years due to ongoing development outside the park. If nomadic males, who are known to range long distances are not able to safely traverse human-dominated landscapes

into areas including Camp Leakey, local males may not be displaced and may not leave natal areas. This may help explain why the number of unflanged subadult males within the study area has gone up slightly since 2006. If these local males are becoming more likely to “stay put”, we may now be picking up on early genetic signals of overall demographic changes within this orangutan population. If genetic changes similar to those documented in Kinabatangan are possibly being detected in a large protected population of orangutans such as that of Tanjung Puting, it is probable that many smaller fragmented and more isolated orangutan populations are also experiencing similar demographic and genetic effects, putting them at a higher risk of extirpation due to deleterious effects of inbreeding and possible lack of breeding opportunities for females.

It is important to note that orangutan rehabilitation/introduction and translocations have also taken place within Tanjung Puting. This influx of “non-local” wild-born ex-captives and translocated individuals, if interbreeding with the local wild individuals, may have had a positive effect on population genetic diversity by increasing heterozygosity, rare alleles, and decreasing overall relatedness. Despite fears (Banes et al. 2016) of deleterious effects from outbreeding and introgression, the risk of this is low between a small number of orangutan individuals from different sub-populations likely having experienced low levels of gene flow in the recent past (Frankham et al. 2011, 2019; Nietlisbach et al. 2012). The low number of alleles and high relatedness within our sample, which includes wild females known for decades and not any known rehabilitants, indicates that the adults in our sample consist of a local wild and interbreeding community and do not represent undetected introduced non-local individuals. It is curious that the number of male encounters has gone down despite the release of ~45 male ex-captive orangutans within the area between 1971-1985 (Galdikas and Ashbury 2013). If these males had stayed within the study area and even some had been mistaken for wild males as they developed cheek pads, encounters should have been higher in 1986 and beyond. Observations on released rehabilitated ex-captive males indicated that virtually all of them, in the manner of wild adolescent/subadult males, wandered and left the study area. For instance, one known released subadult male ex-captive appeared at an OFI/Park outpost approximately 50km from Camp Leakey a year later (Galdikas unpublished data). He did not stay very long. A male offspring of a Camp Leakey ex-captive mother left his natal area and seemed to be permanently residing at another OFI/Park post at least 25 km away. Further investigations into the extent of interbreeding between introduced individuals and the known wild populations are in progress.

Our sample represents just a snapshot of the larger population within the National Park and Tanjung Puting peninsula. Genetic samples analyzed in this study were collected largely over six months from within a core forest area of 7.5 km². The genetic study tried to answer questions relating to the long-term observations of demographic changes. Something happened in the last 50 years. The

number of flanged adult males in the study area decreased in a marked way over a long period of time. As orangutans are long-lived and generation times are wide (Galdikas and Wood 1990), long-term effects of human-caused habitat fragmentation and the direct decreases in population size through poaching/killing on the genetics of remaining wild populations may be slow to develop. But demographic effects move faster. Conservation of orangutans must include both efforts to keep as many individual orangutans alive as possible and to keep intact wild populations thriving. This means not only continuing long-term observational studies and having accurate data on population census sizes but also monitoring the ongoing genetic health of free-ranging orangutan communities and using the valuable information gained to protect more vulnerable at-risk and unprotected remnant populations. It will be difficult, but it will be important to monitor genetic diversity and demographic changes not only within the Camp Leakey population but also in as many disparate remaining orangutan populations as possible. To ensure orangutans as species in the wild continue to be viable, we must maintain habitats and conditions that sustain the behaviors, demographics, and genetics of intact and diverse populations. From the data from the last 50 years at Tanjung Puting it's clear that the situation for male orangutans may be dire. We need to do more to connect, create, and maintain corridors as well as protect larger areas of forest for enabling males to roam successfully as they have over countless previous generations and to help save orangutans from extinction.

ACKNOWLEDGEMENTS

This study would not have been possible without the support of The Indonesian Ministry of Research, Technology and Higher Education (RISTEKDIKTI), the Indonesian Institute of Sciences (LIPI), Directorate General of Nature Resources and Ecosystem Conservation, Indonesian Ministry of Environment and Forestry, Tanjung Puting National Park (TPNP), The University of Palangka Raya, Central Kalimantan, and Center for International Cooperation in Sustainable Management of Tropical Peatland (CIMTROP). BMG especially wishes to thank OFI and their supporters, the Leakey Foundation, and the National Geographic Society and many others for their initial and continued funding of the long-term behavioral and ecological research conducted at Camp Leakey. BMG and the authors especially thank the long-term field staff, including the late Pak Bohap Bin Jalan, associated with Camp Leakey, who helped in sample collection, transport, and processing. REL was funded in part by OFI, Central Washington University (CWU) Graduate and Research Office, Graduate Fellowship from CWU GEAR up, Pete and Sandra Barlow Award and Arlen and Debra Prentice Scholarship. Genetic analysis was made possible by Central Washington University, OFI, and University of Washington Genomics Center. Samples were collected with approved CWU IACUC animal welfare protocol A011523 and all samples were collected with prior

informed consent and permission from the Indonesian government and exported from Indonesia with CITES permit 01152/IV/SATS-LN/2017. The authors wish to thank M. Hart for helpful comments on an early draft of this manuscript. The authors have no competing interests to declare.

REFERENCES

- Arora N, Nater A, van Schaik CP, Willems EP, van Noordwijk MA, Goossens B, Morf N, Bastian M, Knott C, Morrogh-Bernard H, Kuze N, Kanamori T, Pamungkas J, Perwitasari-Farajallah D, Verschoor E, Warren K, Krützen M. 2010. Effects of pleistocene glaciations and rivers on the population structure of Bornean orangutans (*Pongo pygmaeus*). *Proc Natl Acad Sci USA* 107 (50): 21376-21381. DOI: 10.1073/pnas.1010169107.
- Arora N, Van Noordwijk MA, Ackermann C, Willems EP, Nater A, Greminger M, Nietlisbach P, Dunkel LP, Atmoko SSU, Pamungkas J, Perwitasari-Farajallah D, Van Schaik CP, Krützen M. 2012. Parentage-based pedigree reconstruction reveals female matrilineal clusters and male-biased dispersal in nongregarious Asian great apes, the Bornean orang-utans (*Pongo pygmaeus*). *Mol Ecol* 21 (13): 3352-3362. DOI: 10.1111/j.1365-294X.2012.05608.x.
- Banes GL, Galdikas BMF, Vigilant L. 2016. Reintroduction of confiscated and displaced mammals risks outbreeding and introgression in natural populations, as evidenced by orang-utans of divergent subspecies. *Sci Rep* 6: 22026. DOI: 10.1038/srep22026.
- Brend S. 2006. Tanjung Puting National Park, Orangutans and their habitat. Orangutan Foundation and Yayasan, Indonesia. [Indonesian]
- Chesser RK. 1991. Gene diversity and female philopatry. *Genetics* 127 (2): 437-447. DOI: 10.1093/genetics/127.2.437.
- Frankham R, Ballou JD, Eldridge MDB, Lacy RC, Ralls K, Dudash MR, Fenster CB. 2011. Predicting the probability of outbreeding depression. *Conserv Biol* 25 (3): 465-475. DOI: 10.1111/j.1523-1739.2011.01662.x.
- Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P. 2019. A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations. Oxford University Press, England. DOI: 10.1093/oso/9780198783411.001.0001.
- Galdikas BMF, Ashbury A. 2013. Reproductive parameters of female orangutans (*Pongo pygmaeus wurmbii*) 1971-2011, a 40-year study at Tanjung Puting National Park, Central Kalimantan, Indonesia. *Primates* 54 (1): 61-72. DOI: 10.1007/s10329-012-0331-5.
- Galdikas BMF, Shapiro GL. 1994. A Guidebook to Tanjung Puting National Park, Kalimantan Tengah (Central Borneo), Indonesia. PT Gramedia Pustaka Utama and Orangutan Foundation International, Jakarta.
- Galdikas BMF, Wood JW. 1990. Birth spacing patterns in humans and apes. *Am J Phys Anthropol* 83 (2): 185-191. DOI: 10.1002/ajpa.1330830207.
- Galdikas BMF. 1979. Orangutan adaptation at Tanjung Puting Reserve: Mating and Ecology. In: Hamburg DA, McCown ER (eds). *The Great Apes: Perspectives on Human Evolution Volume V* Menlo Park, Calif. The Benjamin/Cummings Publishing Company, San Francisco.
- Galdikas BMF. 1982. Orang-utan tool-use at Tanjung Puting Reserve, Central Indonesian Borneo (Kalimantan Tengah). *J Hum Evol* 11 (1): 19-24. DOI: 10.1016/S0047-2484(82)80028-6.
- Galdikas BMF. 1983. The orangutan long call and snag crashing at Tanjung Puting Reserve. *Primates* 24: 371-384. DOI: 10.1007/BF02381982.
- Galdikas BMF. 1985a. Orangutan sociality at Tanjung Puting. *Am J Primatol* 9 (2): 101-119. DOI: 10.1002/ajp.1350090204.
- Galdikas BMF. 1985b. Adult male sociality and reproductive tactics among orangutans at Tanjung Puting. *Folia Primatol* 45 (1): 9-24. DOI: 10.1159/000156188.
- Galdikas BMF. 1985c. Subadult male orangutan sociality and reproductive behavior at Tanjung Puting. *Am J Primatol* 8 (2): 87-99. DOI: 10.1002/ajp.1350080202.
- Galdikas BMF. 1988. Orangutan diet, range, and activity at Tanjung Puting, Central Borneo. *Int J Primatol* 9: 1-35. DOI: 10.1007/BF02740195.
- Galdikas BMF. 1995. Reflections of Eden, My Years with the Orangutans of Borneo. Little, Brown and Company, Canada.
- Garner A, Rachlow JL, Hicks JF. 2005. Patterns of genetic diversity and its loss in mammalian populations. *Conserv Biol* 19 (4): 1215-1221. DOI: 10.1111/j.1523-1739.2005.00105.x.
- Goossens B, Chikhi L, Jalil MF, Ancrenaz M, Lackman-Ancrenaz I, Mohamed M, Andau P, Bruford MW. 2005. Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan (*Pongo pygmaeus*) populations from Sabah, Malaysia. *Mol Ecol* 14 (2): 441-456. DOI: 10.1111/j.1365-294X.2004.02421.x.
- Goossens B, Setchell JM, James SS, Funk SM, Chikhi L, Abulani A, Ancrenaz M, Lackman-Ancrenaz I, Bruford MW. 2006. Philopatry and reproductive success in Bornean orang-utans (*Pongo pygmaeus*). *Mol Ecol* 15: 2577-2588. DOI: 10.1111/j.1365-294X.2006.02952.x.
- Hamilton RA, Galdikas BMF. 1994. A preliminary study of food selection by the orangutan in relation to plant quality. *Primates* 35 (3): 255-263. DOI: 10.1007/BF02382723.
- Kalinowski ST, Taper ML, Marshall TC. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Mol Ecol* 16 (5): 1099-1106. DOI: 10.1111/j.1365-294X.2007.03089.x.
- Linsky RE, Wagner RS, Djojoasmoro R, Lorenz J, Galdikas BMF. 2022. Cross species use of human microarray genotyping technology for Bornean Orangutan (*Pongo pygmaeus*) SNP discovery. *HAYATI J Biosci* 29 (1): 62-75. DOI: 10.4308/hjb.29.1.62-75.
- Morrogh-Bernard HC, Morf NV, Chivers DJ, Krützen M. 2011. Dispersal patterns of Orang-utans (*Pongo* spp.) in a Bornean peat-swamp forest. *Int J Primatol* 32: 362-376. DOI: 10.1007/s10764-010-9474-7.
- Muir CC, Galdikas BMF, Beckenbach AT. 2000. mtDNA sequence diversity of Orangutans from the islands of Borneo and Sumatra. *J Mol Evol* 51 (5): 471-480. DOI: 10.1007/s002390010110.
- Nater A, Nietlisbach P, Arora N, van Schaik CP, van Noordwijk MA, Willems EP, Singleton I, Wich SA, Goossens B, Warren KS, Verschoor EJ, Perwitasari-Farajallah D, Pamungkas J, Krützen M. 2011. Sex-biased dispersal and volcanic activities shaped phylogeographic patterns of extant Orangutans (genus: *Pongo*). *Mol Biol Evol* 28 (8): 2275-2288. DOI: 10.1093/molbev/msr042.
- Nietlisbach P, Arora N, Nater A, Goossens B, Van Schaik CP, Krützen M. 2012. Heavily male-biased long-distance dispersal of orang-utans (genus: *Pongo*), as revealed by Y-chromosomal and mitochondrial genetic markers. *Mol Ecol* 21 (13): 3173-3186. DOI: 10.1111/j.1365-294X.2012.05539.x.
- Nietlisbach P, Nater A, Greminger MP, Arora N, Krützen M. 2010. A multiplex-system to target 16 male-specific and 15 autosomal genetic markers for orang-utans (Genus: *Pongo*). *Conserv Genet Resour* 2: 153-158. DOI: 10.1007/s12686-010-9278-2.
- Nsubuga AM, Robbins MM, Roeder AD, Morin PA, Boesch C, Vigilant L. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. *Mol Ecol* 13 (7): 2089-2094. DOI: 10.1111/j.1365-294X.2004.02207.x.
- Raymond M, Rousset F. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J Hered* 86 (3): 248-249. DOI: 10.1093/oxfordjournals.jhered.a111573.
- Rousset F. 2008. Genepop'007: A complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour* 8 (1): 103-106. DOI: 10.1111/j.1471-8286.2007.01931.x.
- Utami SS, Goossens B, Bruford MW, de Ruiter JR, van Hooff JARAM. 2002. Male bimaturism success and reproductive success in Sumatran orang-utans. *Behav Ecol* 13 (5): 643-652. DOI: 10.1093/beheco/13.5.643.
- Utami-Atmoko S, Traylor-Holzer K, Rifqi MA, Siregar PG, Achmad B, Priadajati A, Husson S, Wich S, Hadisiswoyo P, Saputra F, Campbell-Smith G, Kuncoro P, Russon A, Voigt M, Santika T, Nowak M, Singleton I, Sapari I, Meididit A, Chandradewi DS, Ripoll Capilla B, Ermayanti, Lees CM. 2017. Orangutan Population and Habitat Viability Assessment: Final Report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, California.
- Van Noordwijk MA, Arora N, Willems EP, Dunkel LP, Amda RN, Mardianah N, Ackermann C, Krützen M, van Schaik CP. 2012. Female philopatry and its social benefits among Bornean orangutans. *Behav Ecol Sociobiol* 66: 823-834. DOI: 10.1007/s00265-012-1330-7.
- Wang J. 2007. Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genet Res* 89 (3): 135-153. DOI: 10.1017/S0016672307008798.
- Wang J. 2011. Coancestry: A program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Mol Ecol Resour* 11 (1): 141-145. DOI: 10.1111/j.1755-0998.2010.02885.x.
- Wright S. 1931. Evolution in mendelian populations. *Genetics* 16 (2): 97-159. DOI: 10.1093/genetics/16.2.97.