Reproductive success and paternity concentration in wild male orangutans (genus *Pongo*)

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Abstract

Due to the unpredictable and extensive ranging behavior of male orangutans, only little is known about the outcome of their reproductive strategies. Unlike other great apes, orangutans do not live in stable social groups, but rather exhibit individual-based fission-fusion sociality. There are two recognizable sexually mature male morphs, which are physically and behaviorally distinct and pursue a different mating strategy. The extent to which males can successfully monopolize the access to fertile females is debated and, due to ecological factors, expected to vary considerably between populations. Monopolization potential and reproductive skew have been so far difficult to estimate from observations alone. We used genetic techniques and long-term behavioral data to reconstruct pedigrees in two orangutan populations located in Sumatra (Suaq Balimbing) and Borneo (Tuanan). Genotyping was performed for 27 microsatellite loci from non-invasively collected fecal samples and we assigned 10 paternities in Suaq Balimbing (N=55 unique individuals) and 8 in Tuanan (N=58 unique individuals). In Suaq reproductive success is skewed and the dominant flanged male obtains more paternities than subordinate flanged males or unflanged males. In Tuanan paternities were assigned to 3 flanged males and one unflanged one. The results of this study suggest that differences in the distribution of reproductive success among males varies between two populations and that monopolization potential for the dominant male in Sumatran populations might be higher than expected. Our study therefore lends insight in to the effectiveness of mating strategies of this elusive great ape.

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1. INTRODUCTION

1.1 Social systems

In most primate species, females mainly compete over food resources while males compete over the access to mates. The availability and distribution of these resources have therefore a significant impact on the patterns of competition and on the social structure of most species (van Schaik 1983). Two types of competition are predicted (Janson and van Schaik 1988); scramble and contest competition. Scramble competition occurs when the limiting resource is highly dispersed in small, low-quality patches or is relatively abundant but its usage cannot be monopolized. In contest competition, the limiting resource is distributed in large, high-quality patches that are easier to monopolize (Wrangham 1979). These types of competition can act on both within-group and between-group levels and will likely affect various aspects of a species social system (Sterk *et al*. 1997).

1.1.1 Female social organization and social structure

Socio-ecological models predict that female social organization is mainly shaped by feeding competition and predation pressure (Fig 1-1, Sterck *et al*. 1997, Janson and Goldsmith 1995). High predation pressures usually favor larger group sizes (Isbel 1994), whereas within-group contest competition for food favors the formation of coalitions, social bonds and steep dominance hierarchies among females. Scramble competition, in contrast, favors the formation of small groups, less steep (or absent) dominance hierarchies and reduced territoriality (Sterck *et al*. 1997). These competitive regimes lead, in the majority of primate species, to two categories of female relationships: Resident-nepotistic and dispersal-egalitarian (Sterck *et al* 1997, Kappeler and van Schaik 2002). Sterck *et al*. tested the solidity of the socio-ecological model for females and found that 27 primate species agree with the predictions.

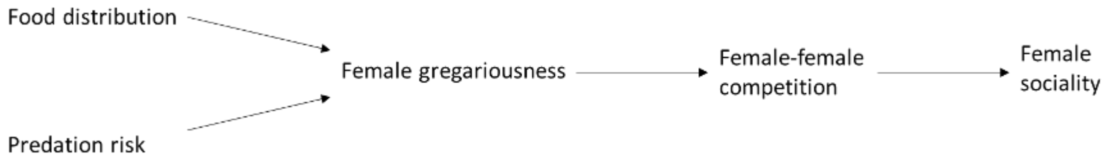

Figure 1-1: Schematic representation of the socio-ecological model for females (Sterck *et al*. 1997)

1.1.2 Male social structure

Food distribution and predation risk determine the distribution of females. Male distribution can also be affected by food distribution and predation pressure (van Schaik 1983). However, intrasexual competition is a major predictor of male distribution (Wrangham 1979). Males compete for the access to a valuable resource that limits their reproductive success. For species in which males do not provide parental care for their offspring, the most valuable resource is the access to fertile females (Wrangham 1979). The type and strength of competition are thus predicted to shape the patterns of male association and the socio-sexual strategies.

An indicator of the strength of male-male competition is the “operational sex ratio (OSR)” (Emlen and Oring 1977, Kvarnemo and Ahnesjö 1996). This measure represents the proportion of fertile females to fertile males. The availability of fertile females at any given time is influenced by several factors, such as the number of females per group, the reproductive rate of females, the degree of breeding seasonality, and the female’s behavior (Clutton-Brock and Parker 1992). In most primate species, the OSR is skewed to females (MItani *et al*. 1996). As OSR becomes more skewed, contest competition between males increases (Mitani *et al*. 1996, Grant *et al*. 1995).

Monopolization potential is a major determinant of primate mating systems (Kappeler and van Schaik 2002) and the OSR can be used to estimate the extent to which males can monopolize the access to fertile females. Factors like the number of males in a group, the level of gregariousness of females and the level of synchrony of their receptive periods are tightly linked to the likelihood of males to maintain exclusive access to females (Ostner *et al*. 2008). When the likelihood of monopolizing the mating opportunities is very high, the predicted social system has highly despotic ranks, intolerance between males and more or less frequent violent takeovers. In contrast, when the access to mates cannot be monopolized, less steep dominance hierarchy and, sometimes, male-male coalitions are expected (e.g. Steenbeek *et al*. 2000, van Noorwijk and van Schaik 2001, Sprague 1992, Takahata *et al*. 1999, Berard 1999).

The differences in mating systems can be reflected in patterns of reproductive success. Paternity concentration (or reproductive skew) represents how reproductive success is distributed among males. In despotic species, paternity is expected to be highly concentrated, and in this case only a small proportion of males is reproductively very successful (e.g. Nsubuga *et al*. 2008), while the majority of males has low or no reproductive success at all. In species with more relaxed dominance among males, several males are successful, with a reduced variability of reproductive success among males (e.g Strier *et al*. 2011, Kutsukake and Nunn 2006 for a review using mating skew).

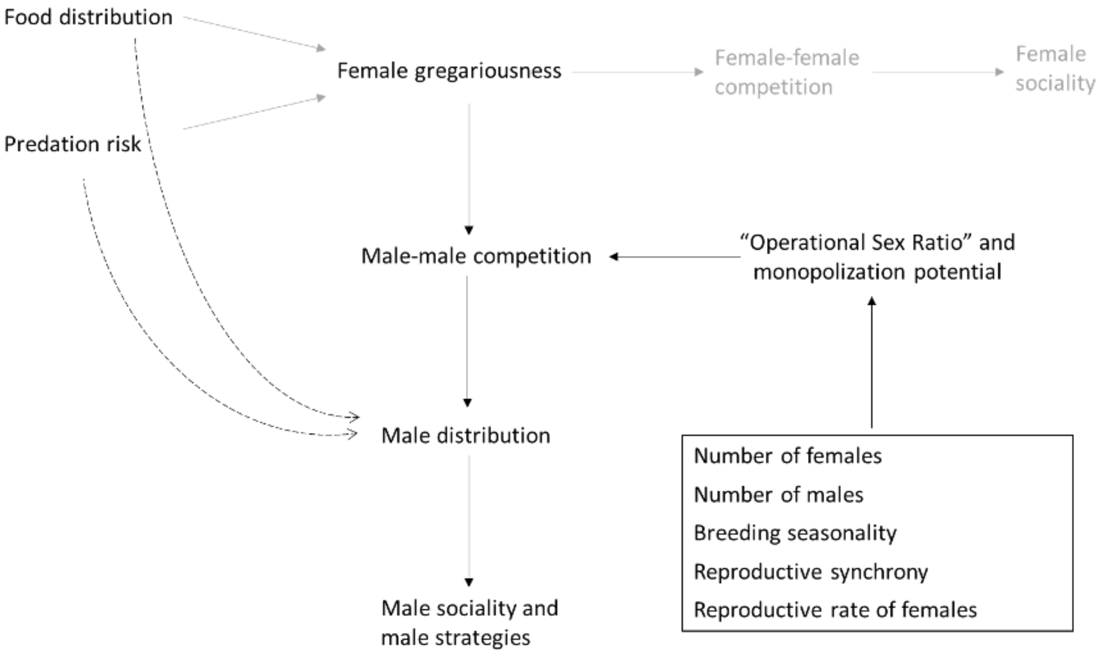

Fig 1-2: Schematic representation of the socio-ecological model for males (adapted from Sterck *et al*. 1997).

1.2 Male mating strategies

Group composition, strength of male-male competition and monopolization potential are factors that influence the reproductive strategies that males employ (Kappeler and van Schaik 2002).

When possible, males try to limit the access to receptive females (e.g. Mass *et al*. 2009, Setchel *et al*. 2005, Watts 1998). In despotic societies, subordinate males try to mate opportunistically (e.g. Matsumoto-Oda 1999, Matsubara 2003), form friendships with females (e.g. Massen *et al*. 2012, Palombit *et al*. 1997) or form coalitions against the top-ranking males (e.g. Highham and Maestripieri 2010).

In more egalitarian societies, sperm competition is a widespread strategy (Møller 1988). Males compete for fertilization by producing large amounts of sperm. This type of competition usually leads to anatomical adaptations, such as large testicles and highly resilient sperm (Anderson and Dixon 2002).

Another factor linked to male-male competition and the evolution of alternative mating strategies in primates, albeit rare, is male bimaturism. Male Mandrills (*Mandrillus sphinx*) show reversible bimaturism that reflects dominance (Dixon *et al*. 1993). Dominant "fatted" males sire most of the offspring in the group and subordinate "non-fatted" males mate opportunistically (Wickings *et al*. 2009). Adult, sexually mature males of long-tailed macaques (*Macaca fascicularis*) are present in two sexually mature forms. The transition to the one with fully developed secondary sexual characteristics is irreversible but also flexible (Not sure what you mean by 'flexible'), with males completing their development following social as well as internal clues (Van Noordwijk and van Schaik 2001).

1.3 Diversity of mating systems in Apes

1.3.1 Genus Hylobates

Within the genus *Hylobates*, white-handed gibbons (*Hylobates lar*) are a highly territorial, pair-bonded, family-living species (Brockelman *et al*. 1998). Siamangs (*Symphalangus spp*.) also live in pairs but have also been observed living in groups with more than two adults with one female and multiple males (Lappan 2007).

Several hypotheses for the evolution of social monogamy in *Hylobates* species have been tested and it has been proposed that the main role of the male in the pair is the protection of the offspring against other males (van Schaik and Dunbar 1990, Borries *et al*. 2011).

Recently, however, the strictness of monogamy has been questioned, as extra-group copulations and partner changes appear to occur more often than previously thought (Reichard and Sommer 1997).

1.3.2 Genus Gorilla

Gorillas (*Gorilla spp*.) form single-male, multi-female groups consisting usually of 8-10 individuals, even though larger groups (up to 40 individuals) have been reported (Doran & Mcneilage 1998). The low levels of sociality and a weak dominance hierarchy among females observed within groups can be explained by the reduced intensity of feeding contest competition among them (Watts 2001).

Female aggregate in order to benefit from the protection of a single dominant male (Watts 1989, 1991, Lucas *et al*. 2010). For this reason, the competition for the role of the local dominant male (the so-called alpha silverback) is extremely high. Selection for large body size and competitive ability in males is reflected in the extreme body size sexual dimorphism as well as in canine size sexual dimorphism (Plavcan 2001). These features suggest that male gorillas evolved those traits in order to outcompete other males. Paternity for the groups is generally very highly concentrated on the local dominant male, even in presence of other silverbacks (Bradley 2004, 2005, Nsubuga 2008).

1.3.3 Genus Pan

Chimpanzees (*Pan troglodytes*) adopt a flexible fission-fusion social system (Lehmann and Boesch 2004). Chimpanzee communities can have between 20 and 150 individuals and are further divided in smaller subgroups as typical for fission-fusion societies (Goodall 1986).

Strong male cohesion and philopatry are observed and within each community. However, both sexes have a dominance hierarchy, with the highest ranking individuals having priority of access to the monopolizable resources (Goodall 1986). High-ranked females can access to superior food resources and this positively affects their reproductive success (Pusey *et al.* 1997).

Several males live in each community and therefore the probability of monopolizing the access to females is reduced (Wroblewski *et al.* 2009). Competition among males is therefore based on a "priority of access" model (Altmann 1962). High-ranking males mate more often with females and subordinate males can attempt to sire offspring through sperm competition by mating opportunistically or by consorting with females (Tutin 1979). Paternity analysis suggests that male success mostly reflects dominance hierarchy (Boesch *et al.* 2006). However, deviation from the "priority of access" model has been reported. Subordinate, young males are more successful than expected, and the extent of this deviation is likely to be linked to the number of competitors, number of simultaneously receptive females, female choice and the length of the periovulatory phase (Wroblewski *et al.* 2009).

Bonobos also live in multi-male, multi-female groups but the dominance hierarchy appears to be more relaxed; females also form strong coalitions and have higher social status than males (De Waal 1995). Promiscuity is the norm and several males mate with the local females. Estrous females tend to be monopolized by high-rank males, even though the only paternity analysis for wild bonobos revealed that most males are reproductively successful. Paternity success for the dominant male was estimated at 0-40% for the highest ranking males (Gerloff 1999). However, due to uncertainty about the dominance status of the males at the time of conception for several offspring, the interpretation of these results is problematic.

1.4 Orangutans: The unique case of the solitary red ape

Unlike gibbons and the African great apes, orangutans (*Pongo pygmaeus and Pongo abelii*) live non-gregariously in individual-based fission-fusion societies (van Schaik 1999, Galdikas 1985). Females show a high degree of philopatry (Arora *et al.* 2012, Morrogh-Bernard *et al.*

2011) and despite their solitary nature, seem to receive social benefits in doing so (van Noordwijk *et al*. 2012). Similar to most mammal species, males tend to leave the natal area once reached sexual maturity, as a response to intrasexual competition and inbreeding risk (Arora *et al*. 2012, Morrogh-Bernard 2011 *et al*., Lawson *et al*. 2007).

In both recognized orangutan species there is flexible and irreversible male bimaturism. Adult, sexually mature males are present in two morphs and it can take 20 or more years after reaching adulthood before the transition. The changes that occur during the irreversible transition from the unflanged morph to the flanged morph are quite drastic, with an increase in body size and the development of secondary sexual characteristics such as more developed throat sacs, wider cheek pads and longer hair on their backs (MacKinnon 1974). Throat sacks and check pads are used by flanged males to emit and precisely direct a long-distance vocalization, the long call (MacKinnon 1974). Long calls are used in social contest to attract mates and to regulate the spatial organization of males (Delgado *et al*. 2009).

1.5 Orangutans: Two species and two distinct mating systems?

In both orangutan species, female orangutans have an exceptionally slow life history (Wich *et al*. 2004, Galdikas and Ashbury 2012) and live at relatively low densities (Husson *et al*. 2009). These conditions generate highly skewed operational sex ratio (Mitani *et al*. 1996), with several males competing over few fertile females. Sexual selection theory suggests that in case of contest competition, the competing sex will evolve traits that enhance competing abilities, such as large size, weaponry, threat signals, female harassment as well as alternative reproductive strategies (Anderson and Iwasa 1996). Orangutans have extreme body size sexual dimorphism as well as strong canine sexual dimorphism (Plavcan and van Schaik 1992, 1997). Both traits are often associated with contest competition and are observed in other primate species that experience strong male-male contest competition (Plavcan and van Schaik 1992).

Field observations strongly suggest that socio-ecological differences between the two species are present and influence the mating system and patterns of competition among males (Delgado and van Schaik 2000, Dunkel *et al*. 2013). In particular, food abundance is a well-documented source of differentiation between the two species (Marshall *et al*. 2009). On Sumatra food availability is higher compared to Borneo and this allows orangutans to live at higher densities (Husson *et al*. 2009, Singleton *et al*. 2009). Females' home range overlap is also more expensive (Singleton *et al*. 2009). Additionally, males and females can associate for longer periods of time and in larger parties (Delgado and van Schaik 2000, Mitra Setia *et al*.

2009). As predicted by socio-ecological models (fig 1-2), food availability and the level of gregariousness of females are major predictors of male-male competition and monopolization potential (Kappeler and van Schaik 2002). It is suggested that the differences in gregariousness affect several components of the two orangutan species' mating systems (Delgado and van Schaik 2000). In order to take on a comparative study of male reproductive success, it is fundamental to understand the differences in the mating system of these species.

1.5.1 The mating system of the Sumatran orangutan (Pongo abelii)

Sumatran orangutans live in rich environments that allow them to have higher levels of gregariousness compared to Bornean orangutans (Husson *et al*. 2009). Party size is also considerably larger (Mitra Setia *et al*. 2009). As females tend to be quite gregarious, a single flanged male can associate with several females for prolonged periods of time (Singleton and van Schaik 2002). It is therefore likely that a male can successfully monopolize the access to several females (Pradhan *et al*. 2012).Field observations suggest indeed that with one single male can dominate over a given territory (Utami *et al*. 2002, Singleton and van Schaik 2002). The dominance hierarchy among males can be very steep and the top ranking male can dominate for over a decade (Utami *et al*. 2002).

The dominant male employs the "sit-and-wait" reproductive strategy (Utami *et al*. 2002). He emits long calls and attract receptive females. This strategy relies on the female preference for flanged males. Playback experiments showed that females indeed approach spontaneous long calls of the dominant flanged male (Delgado 2003, Mitra Setia and van Schaik 2007, Utami and Mitra setia 1995). The fact that even non-receptive females with dependent offspring approach the dominant male's long call suggest that females rely on him for protection (Mitra Setia and van Schaik 2007). In addition, forced copulations involving the dominant male are never observed (Singleton and van Schaik 2002).

Unflanged males employ a different reproductive strategy, the "go-and-search" strategy (Utami *et al*. 2002). Unflanged males cannot produce long calls while roaming the forest in search for receptive females. Roaming unflanged males have been observed copulating, suggesting that the go-and-search strategy might indeed facilitate the access to females. Flanged males end the association between unflanged males and females by chasing the male away (Schürmann 1981, Utami and Mitra-Setia 1995). However, unflanged males, being more agile than flanged males, have never been observed getting injured by flanged males (Utami Atmoko *et al*. 2009a).

It has been suggested that because of the high food availability in Sumatra, the sit-and-wait strategy might only be effective for the dominant male (Pradhan and van Schaik 2012). He can associate with several females for prolonged periods of time and successfully exclude other flanged males. In addition, the local dominant flanged male is more likely to attract females with his long calls (Mitra Setia *et al*. 2009).For subordinate flanged males, the sit-and-wait strategy might also be unsuccessful because long calls advertise the presence to the local dominant male, who is likely to approach other male's calls and chase them away (Utami Atmoko *et al* 2009a).The dominant male is in fact highly intolerant towards other flanged males.

As a result of stable dominance hierarchy, most of the time subordinate flanged males might have extremely low mating chances and negligible reproductive success (Pradhan *et al*. 2012). For subordinate flanged males, the go-and-search strategy could thus represent an alternative mating strategy. However, compared to unflanged males, they require more energy and incur in higher costs when moving because of their size. Due to the presence of tigers, a feared predator, orangutans don't have the option to move on the ground, a far less energetically costly form of transportation (Cant 1987).

With the exception of the dominant male, most males in the population remain unflanged (Dunkel *et al* 2013). The constant presence of a clearly dominant individual might thus induce the developmental arrest. The mechanisms that regulate this developmental arrest are, so far, unknown. Different factors are thought to influence the 'choice' of the optimal strategy. It has been argued that unflanged males use long calls to monitor the presence of flanged males and only become flanged when the probability of becoming dominant are high (Pradhan *et al*. 2012). Behavioral observations show that periods of instability in the dominance hierarchy can indeed promote flanging events (Harrison and Chivers 2007, Utami *et al*. 2002, Pradhan *et al*. 2012). It is then possible that within each population the ratio of males pursuing either strategy reaches an equilibrium in which each males employs the strategy that ensures him the highest chances of reproducing.

1.5.2 The mating system of the Bornean orangutan (Pongo pygmaeus)

Bornean orangutan are more solitary compared to Sumatran ones (Husson *et al*. 2009). Females are highly dispersed and only rarely associate with each other. (Singleton *et al*. 2009). Due to ecological constraints, the association between males and females is only feasible for limited amounts of time (Delgado and van Schaik 2000). This situation is substantially different from what observed in Sumatra. In particular, as a consequence of lower densities and energetic constraints, monopolization potential for one single male is highly reduced (Utami

Atmoko *et al*. 2009b, Delgado and van Schaik 2000).Field observations suggest that dominance hierarchy among flanged males is highly unstable and fights are more common compared to Sumatran populations (Utami Atmoko *et al*. 2009b). In particular, the presence of a clearly dominant male is a rare and short-lived event (e.g. in Tuanan) (van Noordwijk, personal communication). Because prolonged association between one flanged male and the resident females is deemed to be too energetically costly, several flanged males can have access to fertile females (Delgado and van Schaik 2000).

Peri-ovulatory females prefer to mate with flanged males (Knott *et al*. 2010) and estrous females even approach the long calls of flanged males (Spillman *et al*. 2010). In contrast, females with dependent offspring move away from (Spillman *et al*. 2010). In contrast to what observed in Sumatra, flanged males also force copulations, similarly to what unflanged males in both species do (Delgado and van Schaik 2002, Knott *et al*. 2010).

Because flanged males are always dominant over unflanged males (Mitani 1985), and because no single male can successfully monopolize the access to females, it has been suggested that in Bornean populations, unflanged males have a competitive disadvantage (Pradhan *et al*. 2012). Additionally, due to the lack of predators, flanged males in Borneo can also travel on the ground and reduce their energetic expenses, making it easier to find females (Cant 1987). The competitive advantage that flanged males appear to have over unflanged males in Borneo is one of the possible explanations for the reduced (or lack of) developmental arrest. Additionally, the perception of dominance instability might promote flanging events (Dunkel *et al*. 2013, Pradhan *et al*. 2012).

1.6 Previous studies of paternity in orangutans

In order to further study the evolution and maintenance of alternative mating strategies, an estimate of reproductive success is required and this can be assessed with an analysis of paternity. Studies on both Sumatran and Bornean population (Utami *et al*. 2002, Goossens *et al*. 2006) showed that both unflanged and flanged males can successfully sire offspring, thus confirming that alternative mating strategies can be pursued.

In a Sumatran population at Ketambe, paternities for 10 individuals born over a period of almost 30 years were assigned using genetic analysis. Four infants were sired by three flanged males and the remaining six were sired by three unflanged males (Utami *et al*. 2002). Of the four infants sired by flanged males, three were sired by two local dominant flanged males and one by a subordinate flanged male. This suggests that the dominant flanged male has a reproductive advantage over the subordinate flanged males. Of the six offspring sired by unflanged males, three were sired by a single individual, who later became the local dominant flanged male. Since the number of flanged males and the number unflanged males were roughly the same over time, the authors suggest that reproductive success for flanged and unflanged males is similar, but a few aspects should be considered;

In this population wild-born, ex-captive females were introduced and this might have influenced the paternity analysis. Two of the three infants that one unflanged male sired, were conceived with these wild-born, ex-captive females (or daughters of ex-captive), which could potentially display different sexual behaviors or developmental trajectories from the ones commonly observed for native females (Utami Atmoko *et al*. 2009b). At least four of the six offspring sired by unflanged males were the first offspring for the mother, suggesting that flanged males prefer more experienced females and might consent unflanged males to mate with younger, nulliparous females, who also seem to voluntarily interact with unflanged males (Utami Atmoko *et al*. 2009b).

A similar study conducted in a Bornean population (Goossens *et al*. 2006) investigated paternity of 16 individuals and assigned it to 10. Nine of them were fathered by five flanged males and one from an unflanged male. Among the flanged males paternity was distributed as follows: One fathered three infants over a time span of 15 years (1985,1998,2000), a second male fathered two infants (both in 1990), another male fathered two infants (1990, 1999) and two more fathered one infant (both in 1990). In contrast with the previously described study (Utami 2002), in this area unflanged males appear to be far less successful than flanged males.

Even though these studies don't provide conclusive evidence about the outcome of different reproductive strategies and the differences between the two orangutan species, they provide a basis on which I can formulate the hypotheses for my study.

1.7 Paternity assignments in wild animal populations

Assignment of paternity in wild populations has always been a difficult process. On one hand, in most mammal species, maternity can be assigned using behavioral observations, since pregnancies can be monitored and the offspring is often seen in contact with its mother. A reliable paternity assignment, on the other hand, requires more than just behavioral data. The distribution of individuals and the mating history of the mother can help identifying potential fathers but certainty of paternity is, nowadays, only acquired using genetic data (Jones and Ardren 2003).

The advent of genetic data prompted the development of methods that can be used to assign paternities. The first methods, used in humans, were based on exclusion criteria (Chakraborty 1974). These methods rely on the genetic incompatibility between offspring and candidate fathers and aim at excluding as many potential fathers as possible from the pool of candidate fathers until only one is not excluded. One limitation of this method is that if the number of potential fathers is high or the power of the markers used not sufficiently high, it's not always possible to reduce the list of non-excluded fathers to only one individual.

A second methodology, based on likelihood scores (Thompson 1975, Meagher 1986), aims at assigning the most likely father to the offspring. For each candidate father, two hypotheses (H1: candidate is the real father, H0: candidate is not the father) are tested and a likelihood ratio is calculated. A large likelihood ratio means that H1 is more likely than H0 and therefore the candidate with the highest ratio is considered to be the true father. Assignment can be either categorical or fractional; in the first case the offspring is assigned to one potential father, while in the second case paternity will be distributed proportionally among all the potential fathers according to the likelihood scores. Whereas the fractional assignment doesn't appear to have any biological relevance, it has been observed that for the testing of particular hypotheses, it can have more statistical power than the categorical approach (eg Devlin 1988, Smouse and Meagher 1994). Unlike the exclusion approach, a likelihood-based assignment requires statistical validation and simulated population-specific likelihood ratios based on allele frequencies allow assignment of paternity within any desired confidence interval (Marshall *et al* 1998). Statistical support is needed because the presence of relatives of the true father or relatives of the offspring (first cousins, half siblings, full-siblings) can lead to high likelihood scores and thus to false assignments. Tests revealed that the presence of males that are closely related to the offspring among the potential fathers can indeed cause a reduction of the confidence of the assignment. Krützen *et al*. (2004) addressed the problem using a methodology developed by Brenner and Weir (2003) and incorporated in his analysis a likelihood ratio for the half-sibling and the true father hypotheses, allowing him to quantitatively assess whether a parent-offspring relationship was more likely than a half-siblings one (or vice versa).

Many software packages have been successfully used for paternity assignment in natural populations (see Jones and Ardren 2003 for a list). For orangutan populations, the software Cervus 3.0 (Marshall *et al* 1998, Kalinowsky 2007) has been proven to be a reliable choice (Utami *et al*. 2002, Goossens *et al*. 2006).

Another interesting solution is offered in the software COLONY 2.0 (Wang 2012). Instead of using pairwise comparisons, like most other programs (including Cervus 3.0) do, COLONY 2.0 implements full pedigree likelihood comparisons which test the likelihood of the whole pedigree rather than just test dyadic relationships. A comparison of the two programs (Karaket 2012) revealed that COLONY 2.0 required fewer markers to reach the desired level of accuracy, with the only drawback of necessitating up to three days to perform one single analysis (opposed to the 30 minutes required by Cervus 3.0).

2 HYPOTHESES

The goal of my study is to investigate the reproductive success of male orangutans. Three main aspects are examined:

1) Paternity Concentration:

In species in which a dominance hierarchy among males is established it is often observed that the highest ranking individuals sire most of the offspring in their populations (e.g. Bradley 2004, Boesch *et al* 2006). Among male orangutans strong male-male contest competition for the access to females is observed and a dominance hierarchy among them is established (Utami *et al* 1997). Assessing how paternity is distributed in wild orangutan populations can help understanding to which extent males can monopolize estrous females.

Paternity concentration can be estimated by assessing the variance of male reproductive success in the population. In wild populations is not always possible to collect samples from all the potential fathers and therefore for a portion of the sample it is not possible to successfully identify the father (e.g. Goossens *et al.* 2006). In order to overcome this issue and enlarge the sample size it is possible, by looking at the number of paternal half siblings in a population, to assess whether offspring for which the fathers wasn't successfully identified have the same father or have different ones. In my analysis I will use this approach to try to assess paternity concentration in my study's populations. Predictions are presented in table 2-2.

2) Reproductive success of the two male morphs

Both unflanged and flanged males have been proven to successfully sire offspring in wild orangutan populations (Utami *et al.* 2002, Goossens *et al.* 2006) but it is not known yet whether the two morphs are equally successful or not. I will combine the results from the paternity analysis with the field observations to try to estimate pro capita reproductive success values for both morphs. Predictions are presented in table 2-2.

3) Interspecific differences

Based on field observations, differences between the two species might be reflected in their mating systems and an expected outcome are summarized in Table 2-1.

Table 2-1: Comparison of the two orangutan's species: Relationship between ecology, social- and mating systems. (Adapted from Wich *et al* (2009) and Delgado and van Schaik (2000))

	Sumatra	Borneo	References
Habitat productivity	High quality food all year round	High quality food is scarce and not always available. Orangutans have to rely on low quality food.	Marshall 2009
Ecological constraints to association	Virtually absent. High food availability allows animals to live at higher densities and associate more often	Association and living at high densities is not energetically sustainable due to food scarcity	Husson 2009
Association with local dominant male	Prolonged	Short; longer association is not energetically feasible.	Mitani 1991, Utami 2000
Mating chances for low-ranking flanged males	Low, females are protected by the dominant male	High, females don't have protection and are susceptible to harassment	Utami 2000
Monopolization potential for dominant flanged male	High	Low	Delgado and van Schaik 2000
Arrested development	Prolonged, affects a high proportion of males.	Short or even potentially absent.	Dunkel 2013
Occurrence of forced mating	Low, very rarely involves flanged males	High, often involves also flanged males	Miitani 1985, Galdikas 1985
Concentration of paternity	**Only a small number of males has access to females. Paternity concentration is expected to be high**	**Several males have access to females. Paternity concentration is expected to be low**	

Table 2-2: Expected outcomes of the paternity analysis for the two orangutan species

	CONCENTRATION OF PATERNITY	SUCCESS OF THE TWO MORPHS
BORNEAN ORANGUTAN	Low, only few paternal half siblings	High pro-capita success for flanged males. Low success for unflanged males
SUMATRAN ORANGUTAN	High, elevated presence of paternal half- and full siblings	High success for the dominant flanged male, very low success for subordinate flanged males. Unflanged males only successful with nulliparous females.

3 METHODS

3.1 From fecal sample to ready-to-use DNA

3.1.1 Sampling

Sampling was conducted in two field sites in Indonesia, Suaq Balimbing and Tuanan, located in the northern part of Sumatra and Central Kalimantan, respectively. Suaq Balimbing is located in the western coastal plain of the Leuser and studies of wild orangutans initiated in 1994 but had to be interrupted in 1999 due to political upheaval. In 2007 researchers have started to work again in this region. The Tuanan Orang-utan Research Area is located in the south-eastern part of Kalimantan on Borneo, Indonesia, in peat swamp forest that covers approximately 7.5 Km^2. Studies on wild orangutans have been carried out in this area since 2003.

At both sites, behavioral data have been collected based on a standardized methodology (http://www.aim.uzh.ch/Research/orangutannetwork/FieldGuidelines.html) and feces collection for genetic analysis is performed following standard protocols (http://www.aim.uzh.ch/Research/orangutannetwork/GeneticSamplingProtocol.html). Over the years, samples were collected in using both the Ethanol-based and the silica-based methods. All samples were stored at -20°C immediately after arrival in Zürich in other to prevent further DNA degradation.

3.1.2 Extraction

I extracted DNA from fecal samples using the QIAgen mini Stool Kit following the manufacturer's instruction with the following modifications:

Incubation time of sample in buffer ASL was modified. For very dry samples, especially silica dried ones, vortexing for one minute was not enough to ensure optimal homogenization of the sample with the buffer and therefore increased the incubation time to up to 24 hours. Elution volume was reduced to 100µl and performed in two steps (2x50 µl) with prolonged incubation time before both centrifugation steps. After extraction, the extracts were stored in a fridge until further use.

3.1.3 DNA quantification

For this step, I have used a protocol that was developed by Morin *et al*. (2001) and that has already been adapted for use with orangutan (Morf, MSc thesis 2009).

Table 3-1: Reaction setup for qPCR

Reagent	Concentration	Final concentration	Per reaction [µl]
Template [ml]			2 (5 for standards)
qPCR cmyc F (sigma)	10 µM	0.9 µM	1.8
qPCR cmyc R (sigma) [µM]	10 µM	0.9 µM	1.8
cmyc probe[mM]	10 µM	0.25 µM	0.5
TaqMan Master Mix (AB) 2x	2	1	10
BSA		0.4 µg/µl	0.8
ddH2O			3.1
Final volume			20

Table 3-2: Reaction profile for qPCR

Step:	Temperature [°C]	Time [min:sec]	No. of cycles
Initial Denaturation	95	10:00	
Denaturation	95	00:15	55
Annealing	59	00:30	

Ct values were estimated by the proprietary software (AB StepOne Sofware v2.3) and a standard curve was produced using the Ct values of the standards and their initial concentration. Since the standard curve was then used to estimate the initial DNA concentration in the extracts, I decided to consider only runs with curves with a correlation coefficient (R^2) higher than 0.95. This ensures a reliable estimate of DNA concentration. I amplified each extract and standard twice and then averaged the two DNA concentration estimates.

3.2 Microsatellite genotyping: From DNA to genetic data

For this study I used 28 microsatellite loci (see table 8-1), divided in 5 multiplex setups.

3.2.1 PCR Multiplex

I carried out PCRs in 96-well plates (ABGene SuperPlate 2400) in 8 µl volume. I performed the PCRs using an AB Verity machine and the reaction profile is described in table 3-4.

Table 3-4 Reaction setup for PCR

Reagent	Concentration	Final concentration	Per reaction [µl]
Template [ml]	-	-	1
Primer Mix[1]	10 µM	1 µM	0.8
Multiplex MM	2x	1x	4
ddH2O	-	-	2.2
Final volume			8

[1] Primer mixes were prepared independently for each multiplex

Table 3-5: Reaction profile for PCR

Step:	Temperature [°C]	Time [min:sec]	No. of cycles
Initial Denaturation	95	15:00	1
Denaturation	94	00:30	45
Annealing	58	01:30	
Extension	72	01:00	
Final extension	60	30:00	1

I diluted 1 µl of PCR product 20 to 30 times in pure water and then loaded on a 96-well sequencing plate, along with 10ul of HIDi and 0.07ul of size standard. I denaturated the double-stranded, diluted PCR products for four minutes at 95°C using an AB Verity machine. I then sequenced the single-stranded DNA fragments using a 3730xl DNA Analyzer (Applied Biosystems).

3.2.2 Allele scoring

I scored the amplified alleles using the software GeneMapper v4.0 (Applied Biosystem). GeneMapper v4.0 allows to automatically score alleles but for each reaction, I checked every call, removed wrongly assigned alleles and added true alleles that were not called by the software. This evaluation of the quality of the software-made calls was subjective and based on my personal experience with microsatellite scoring and my familiarity with the loci. I then exported the called alleles in text file and continued my analysis in Microsoft Excel.

3.2.3 Consensus MACRO

This is the final step of the data acquisition process. It is aimed at obtaining a consensus genotype for each extract. The number of times a genotype has to be observed before accepting it depends on the initial DNA concentration in the extract (Table 3-6, based on Morin *et al.* 2001).

Table 3-6: Conditions for the acceptance of genotypes based on Morin et al. (2001)

DNA Concentration (pg/µl)	Heterozygous genotype	Homozygous genotype
0-25	Do not use	Do not use
25-100	Both alleles appear at least twice	Only one allele, observed at least 7 times
100-200	Both alleles appear at least twice	Only one allele, observed at least 4 times
200+	Both alleles appear at least twice	Only one allele, observed at least 2 times

3.2.4 Identity analysis

After the acquisition of a genotype from a newly analyzed sample I checked whether that genotype was already present in the database. To do so, I compared the genotype for five loci that ensure a high level of allelic diversity and thus a low probability of identity. I performed this analysis using the software Cervus 3.0 (Marshall *et al.* 1998). Initially, in order to pick up genotyping mistakes I have allowed the software to indicate all the matches with up to one mismatch. When I observed only one mismatch, I re-analyzed the raw data in GeneMapper v4.0 in order to identify potential genotyping mistakes.

I combined matching genotypes and used them to produce one unique genotype

3.2.5 *Usefulness of genetic markers*

Error rates can be estimated using repeated genotyping of randomly chosen individuals by dividing the number of mismatches for the total number of comparisons (Bonin et al 2004). This approach allows to estimate overall error rates but also locus-specific error rates. I tested each locus for Hardy-Weinberg disequilibrium and each locus pair for Linkage disequilibrium. I carried out this analysis in Genepop v4 (Rousset 2008) and Genalex 6.5 (Peakall and Smouse 2012). After the preliminary tests for Hardy-Weinberg disequilibrium and linkage disequilibrium, for each population I estimated several descriptive parameters using Cervus 3.0 (Marshall 1998, Kalinowski 2007). These parameters offer an overview of the markers used in the study and allow to estimate the power of the dataset. In particular, the number of allele per locus (NA), expected (HE) and observed (HO) heterozygosity, Polymorphic Information Content (PIC) and probability of identity for both unrelated individuals (Pi) and siblings (Pis) are measured in Cervus 3.0.

In order to further estimate the suitability of the genetic markers, I calculated the Power for relationship inference (PW_R) using KinInfor 1.0 (Wang 2006). PW_R indicates the likelihood of the set of loci to discriminate between two pedigree relationships and it is a reliable measurement for the assessment of multilocus informativeness (Wang J, personal communication). For my analysis I tested the ability of my dataset to discriminate between Parent-offspring/unrelated, half-sibling/unrelated, half-sibling/first cousin and Parent-offspring-Full sibling.

3.2.6 *Parentage analysis*

I assigned paternities using the software Cervus 3.0 and COLONY 2.0. All males in the dataset were included as potential fathers with the exclusion of pre-dispersal males. Paternities were assigned at the 80% confidence level. An important parameter for paternity analysis is the proportion of sampled fathers and this value is particularly difficult to estimate in orangutans because of the size of the study sites and the unpredictable ranging patterns of males. The percentage of sampled males was estimated using the number of genotyped males and the number of males observed in the field (Dunkel *et al* 2013). I set this value at 25% for both populations.

I carried out a maternity analysis for all individuals in the dataset. I included every adult female in the pool of potential mothers and the analysis was performed using the software Cervus 3.0 and COLONY 2.0. For each candidate offspring, females that are known to be younger than the offspring were excluded from the list of candidate mothers. This step, although not strictly necessary, can increase the power of the analysis in presence of highly related individuals. Maternities were accepted at the 80% confidence level. The proportion of mothers sampled was estimated based on field observations and on the number of individuals sampled.

3.2.7 Developmental status of the father and reproductive history of the mother

Developmental status of the males were assigned using field observations (information provided by Carel van Schaik and Maria van Noordwijk). In order to assess the reproductive history of the mother I have used the results of my maternity analysis and field observations. In some instances I could not assess with certainty whether the mother was nulliparous or multiparous because, due to strong male dispersal, I have almost only identified female offspring and have no way of identifying previous male offspring that females could have had.

3.2.8 Estimate of paternity concentration

In order to estimate the number of fathers present in the study populations, I used the software COLONY 2.0 (Wang 2012). Even in absence of the real fathers, the software can calculate the confidence for the assignment of dyads of full- or half-siblings. This procedure will be used to estimate paternity concentration by assessing the number of successful males at any given time without requiring the sampling of all the true fathers. I accepted half-sib pair that were assigned at a confidence level of 80%. After assessing the number of fathers in the population I estimated the level and significance of reproductive skew among males. I have done that only for Suaq and for the time period 1988-1997. I calculated the B-index (Nonacs 2000) using Skew Calculator 2013 (Nonacs 2003). The B-index estimates the strength and significance of reproductive skew as follows:

$B<0$	Reproductive success is equally shared
$B=0$	Reproductive success is randomly distributed among males
$B>0$	Reproductive success is skewed and not equally shared

For the data regarding male numbers and presence, I have used the data present in Dunkel *et al*. (2013). Presence estimates were divided in three categories: 50%, 25% and 5%. In order to obtain a conservative estimate of paternity concentration, unknown fathers were assumed to belong to the 25% presence category.

4 RESULTS

4.1 Suaq

Paternity analysis in Cervus 3.0 revealed 10 father-offspring pairs (Table 4-1). 9 out of 10 paternities were assigned to offspring of known mother. In all cases, the confidence for the mother-father-offspring trio reached 95%. Paternities were assigned to 6 different males. Arno obtainined 4 paternities, Ria obtained 2, Mack, Bestel, Dian and Sumbing each obtained 1 paternity.

Table 4-1: Paternity assignments with Cervus 3.0 for Suaq (L_C= Number of loci compared, L_{MM}= Number of mismatches, confidence: *=95%, +=80%)

Offspring ID	Mother ID	Father ID	L_c	L_{MM}	Pair ΔLOD	Pair confidence	Trio ΔLOD	Trio confidence
ROBBI	LENA	ARNO	22	0	10.8	*	13.5	*
ELLIE	FRISKA	ARNO	22	0	4.59	+	9.41	*
LISA	CISSY	ARNO	22	0	4.69	+	13.1	*
TINA	RAFFI	ARNO	22	0	9.53	*	15.3	*
NIBLA	INTAY	BESTEL	21	0	7.88	*	11.1	*
DODI	CHICK	DIAN	21	0	6.13	*	13.6	*
UNO	BIB	MACK	21	1	2.45	+	6.75	*
CHINDY	CISSY	RIA	23	1	5.23	*	10.8	*
LILLY	LISA	RIA	23	0	7.36	*	10.0	*
PRECILLA	na	SUMBING	22	0	5.62	*	na	na

Paternity analysis in COLONY confirmed the paternity assignments obtained with Cervus 3.0. All but one assignments reached the 95% and one assignment reached and the 80% confidence level. No paternal half-sib pair were identified at the 80% significance level (table 4-2). Table 4-2 also includes the information about the year of conception, father's developmental stage (unflanged or flanged), father's rank (dominant or subordinate) and the mother's reproductive history (nulliparous vs multiparous).

Table 4-2 Paternity assignments and Half-sibship assignments for Suaq with COLONY 2.0

Offspring ID	Mother ID	Father ID	Confidence level	Year of conception	Father's status at conception	Father's Rank	Mother's reproductive history
LILLY	LISA	RIA	1.000 (*)	2001	Unknown	UNK	NP
UNO	BIB	MACK	1.000 (*)	1988	Flanged	UNK	UNK
LISA	CISSY	ARNO	1.000 (*)	1988	Flanged	D	UNK
TINA	RAFFI	ARNO	1.000 (*)	1996	Flanged	D	NP
NIBLA	INTAY	BESTEL	1.000 (*)	1996	Unflanged	S	NP
ROBBI	LENA	ARNO	1.000 (*)	1996	Flanged	D	NP
DODI	CHICK	DIAN	1.000 (*)	1991-92	Unknown	S	UNK
CHINDY	CISSY	RIA	0.997 (*)	2002	Unknown	UNK	MP
ELLIE	FRISKA	ARNO	0.986 (*)	1998	Flanged	D	MP
PRECILLA	UNKN_1	SUMBING	0.905 (+)	Unknown	Unknown	UNK	-
SHERA	CHICK	UNKN_1		1997	-	-	MP
FREDDY	FRISKA	UNKN_2	Half-sib: 0.703 (-)	2004	-	-	MP
BARBARA	UNKN_2	UNKN_2		Unknown	-	-	-
ABBY	UNKN_2	UNKN_3	Half-sib: 0.117 (-)	Unknown	-	-	-
HERDY	FRISKA	UNKN_3		1988	-	-	MP
HALTE	FRISKA	UNKN_4		Unknown	-	-	UNK
TEDI	HALTE	UNKN_5		1996	-	-	MP
LENA	DIANA	UNKN_6		1982	-	-	-
DIDDY	DODI	UNKN_7		2005	-	-	MP
NUK	-	UNKN_8		2006	-	-	-
GANI	UNKN_3	UNKN_9		Unknown	-	-	-
RAFFI	HALTE	UNKN_10		Unknown	-	-	UNK

The B-index for the period 1988-1997 was positive and estimated at 0.0464, indicating significant (p=0.048) reproductive skew (see Table 8-8).

4.2 Tuanan

Paternity analysis in Cervus 3.0 revealed 8 father-offspring pairs (Table 4-5). Paternities assigned to individuals with known mothers reached the 95% for the trio confidence level, while one paternity for an individual without known maternal genotype reached 80% confidence level. Paternities were assigned to 4 males, Kentung, Guapo, Niko and Wodan.

Table 4-3: Paternity assignments with Cervus 3.0 for Tuanan (L_C= Number of loci compared, L_{MM}= Number of mismatches, confidence: *=95%, +=80%)

Offspring ID	Mother ID	Father ID	L_c	L_{MM}	Pair ΔLOD	Pair confidence	Trio ΔLOD	Trio confidence
IDO	INUL	GUAPO	10	0	4.73	*	8.17	*
JERY	JINAK	NIKO	18	0	6.80	*	13.2	*
MILO	MINDY	NIKO	20	0	8.90	*	13.7	*
STREISEL	SIDONY	WODAN	25	0	10.9	*	14.4	*
PUMUCKL	PINKY	GUAPO	10	1	1.40	+	2.51	*
LOLO	SIDONY	KENTUNG	23	0	9.48	*	13.1	*
NANIO	-	KENTUNG	23	2	2.76	+	-	-
TIKUS	TALIA	KENTUNG	25	1	10.2	*	16.4	*

A paternity analysis in COLONY confirmed the paternities assigned with Cervus 3.0. One paternal half-sib pair was identified at the 95% confidence level (table 4-4). Table 4-4 also includes the information about the year of conception, father's developmental stage (unflanged or flanged), father's rank (dominant or subordinate) and the mother's reproductive history (nulliparous vs multiparous). Four paternities were assigned to flanged males and one to an unflanged male. In three instances, I could not reliably assess the developmental status of the father.

Table 4-4: Paternity assignments for Tuanan with COLONY 2.0

Offspring ID	Mother ID	Father ID	Confidence level	Year of conception	Father's status at conception	Father's Rank	Mother's reproductive history
JERY	JINAK	NIKO	1.000 (*)	2002	Flanged	?	MP
MILO	MINDY	NIKO	1.000 (*)	2001	Flanged	?	Unknown
STREISEL	SIDONY	WODAN	1.000 (*)	2002	Unflanged	?	MP
IDO	INUL	GUAPO	0.898 (+)	2001	Flanged	?	MP
PUMUCKL	PINKY	GUAPO	0.896 (+)	2001	Flanged	?	Unknown
LOLO	SIDONY	KENTUNG	1.000 (*)	~1995	Unknown	?	Unknown
NANIO	-	KENTUNG	0.997 (*)	1997	Unknown	?	Unknown
TIKUS	TALIA	KENTUNG	1.000 (*)	1999	Unknown	?	Unknown
CIKIPOS	WILMA	UNKN_1	Full-sib: 0.574 (-)	Unknown	-	-	Unknown
FRODO	WILMA	UNKN_1		1997	-	-	Unknown
DESY	INUL	UNKN_2	-	Unknown	-	-	Unknown
JIP	JUNI	UNKN_3	-	2006	-	-	NP
JUNI	JINAK	UNKN_4	-	1994	-	-	MP
KERRY	JINAK	UNKN_5	-	Unknown	-	-	Unknown
KONDOR	KERRY	UNKN_6	-	1999	-	-	Unknown
MINDY	JINAK	UNKN_7	Half-sib: 0.963 (*)	Unknown	-	-	Unknown
WILMA	UNKN_1	UNKN_7		Unknown	-	-	-
SUSI	WILMA	UNKN_8	-	2003	-	-	MP
TALIA	-	UNKN_9	-	Unknown	-	-	-

5 DISCUSSION

In my thesis, I explored the patterns of male reproductive success in a population of Sumatran orangutans (*P. abelii*) and one of Bornean orangutans (*P. pygmaeus wurmbii*). I assigned 10 paternities in the Sumatran population and 8 in the Bornean one. Additionally, I have managed to provide evidence for high paternity concentration for the Sumatran population.

5.1 Reproductive success of male orangutans

5.1.1 Suaq

In Suaq a flanged male, Arno, sired four of six sampled offspring that were born between the late 1980s and the late 1990s. He was reported to be the undisputed dominant male between 1994 (when the observations started) and 1999 (Singleton and van Schaik 2002). I confirmed that at least three of the offspring born around 1996 were indeed sired by Arno. The fact that he also sired at least one of the six offspring born in 1988 suggests that his tenure might have started in the late 1980s.

Between 1996 and 1997, at least 9 offspring were born and Arno sired three of the five infants analyzed in this study. This suggests that paternity concentration might be high, reflecting a high monopolization potential. Based on field observations, this result is not surprising, because Arno was consistently seen in consortship with females. The calculation of the B-index (Nonacs 2003) confirms that male reproductive success in Suaq for the period 1988-1997 is significantly skewed and concentrated in the local dominant male.

The paternity obtained by Mack in 1988 should not be too surprising as he was one of the only three flanged males who regularly ranged within the study area (Singleton and van Schaik 2000). It is even possible that in the late 80s he was the local dominant male.

In 1996, Bestel was unflanged and his success with a young, nulliparous female (Intay) is consistent with what observed in another population of Sumatran orangutans. Schürmann (1982) noted in Ketambe that unflanged males were the first ones to show interest for a young female. Whereas she showed a clear interest in the local dominant male, he did not reciprocate and started to be interested in her only the year before she conceived (Schürmann 1982). It is then possible that if females get pregnant when still in the phase of voluntary courtship with unflanged males, the paternity of the offspring might be acquired by one of these unflanged males (Utami Atmoko 2009b).

Around the end of 1999 Arno was challenged by several immigrant flanged males who entered the area following extensive logging in neighboring forest patches (van Schaik, personal communication). In 2001 and 2002, paternity of two offspring was assigned to Ria, a male who up to 1999 was unflanged. Ria's success with one of the older resident females, Cissy, is a clear sign of the weakening of Arno's dominance (or his disappearance). Cissy and Arno were in fact seen to be often in association throughout the 1990s and already had a daughter together in 1988.

Subordinate flanged males and unflanged males appear to be equally (un)successful and are significantly less successful than the dominant flanged male. When the dominance hierarchy is stable (most of the time), unflanged males might be more successful than subordinate flanged males. Overall, flanged males (dominant and subordinate) are more successful than unflanged males. As unflanged males represent the majority of males in the area (Dunkel *et al* 2013), I suggest that their pro capita reproductive success is low, despite the high levels of sexual activity observed (Utami Atmoko *et al*. 2009b).

My results are partially discordant with the findings for another Sumatran population, Ketambe, in which the dominant males lost several paternities to unflanged males. In that case, one male dominated for approximately 18 years but only obtained one paternity out of four that were investigated when he was present, losing three to two unflanged males (Utami *et al*. 2002). The differences between the two results could be caused by the sexual behavior of the several nulliparous females. These females descend from a line of ex-rehabilitant females and were likely to have developed earlier do to the provisioning of high quality food from humans (Utami Atmoko *et al*. 2009). These females might have gotten pregnant in the phase of voluntary courtship with unflanged males.

5.1.2 Tuanan

For Tuanan, 8 paternities out 18 investigated offspring were assigned. One male of unknown developmental status obtained three paternities, two males (likely to be flanged at the time of conception) sired two offspring and one paternity was assigned to an unflanged male. The three offspring sired by Kentung were born in the mid- and late nineties, while the remaining five offspring were all born between 2001 and 2003, supporting the idea that in this population several males are mating with receptive females.

The relatively low rate of paternity assignment might be a reflection of the high number of transient, non-local males observed in the field. Flanged males come and go and only rarely "settle" in the study area (Dunkel *et al* 2013). Even though one of the frequently present males, Niko, was successful at least twice, it seems that also several transient males might be reproductively successful.

The success for one unflanged male suggests that these males are copulating with estrous females. In another Bornean site, Gunung Palung, the majority of copulations for periovulatory females involved flanged males, but a copulation between a periovulatory female and an unflanged male was observed (Knott *et al*. 2010). In this case the copulation was forced and this observation is compatible with the go-and-search strategy that unflanged males are believed to employ in Borneo.

Based on my results, the hypothesis that in *P. pygmaeus* populations several flanged males are successful whereas unflanged males are only rarely successful cannot be rejected, but more data is needed. An estimate of reproductive skew was not possible to achieve. Most offspring in my sample were born before research started in Tuanan and thus information about male presence is lacking.

5.2 How do these results improve our understanding of orangutan's mating system?

5.2.1 Interspecific differences

My results suggest that the levels of paternity concentration in the two species differ. As predicted, the dominant male in Suaq acquired the majority of the paternities during his tenure, whereas in Tuanan several flanged males are successful at the same time. However, Kentung had high reproductive success in a 5 year window, suggesting that some individuals can be more successful and that paternity may only be concentrated in a few individuals.

Unflanged males are successful in both populations and this result is not surprising for the Sumatran population. In contrast, unflanged males in Borneo were thought to be very unlikely to mate with receptive females (Knott *et al*. 2010) and the success of a Bornean unflanged male raises new questions about their reproductive strategies.

5.2.2 Reproductive success of the unflanged male

Both mine and previous results (Utami 2002), suggest that Sumatran unflanged males might be particularly successful with young nulliparous females. In primate species, male choice for older females might be adaptive (Anderson 1986). Extensive offspring care is required and more experienced females have higher chances of successfully raising an offspring (Paul 1998, Anderson 1986). Additionally, nulliparous females might require more cycles to get pregnant (Gesquiere *et al*. 2007).

In chimpanzee (*Pan troglodytes*), the top ranking males have priority of access to females, and subordinate males may attempt to achieve paternities by mating opportunistically. Each low ranking male can sire between 0% and 10% of the offspring in the group. Younger, low-ranking males only sire the offspring of younger females (Wroblewski 2009, Muller *et al*. 2007).

In a multi-male mountain gorilla (*Gorilla beringei*) group, subordinate silverbacks were found to be more likely to copulate with nulliparous females than the alpha male. In this case, all the nulliparous females were known (or very likely) to be the daughters of the alpha silverback (Stoinski *et al*. 2009). Another study on mountain gorilla, the subordinate males achieve approximately 15% of the paternities and in this case it was suggested that their success is caused by a lack of control by the dominant silverback (Bradley *et al*. 2005).

In baboons (*Papio cynocephalus*), adolescent females conceive in only 1% of the cycles (in contrast to 30% for adult females). Alpha males also in this species mate preferentially with older females (Gesquiere *et al*. 2007). In rhesus macaques (*Macaca mulatta*), adolescent females present sexually more often to low-rank males. Compared to adult females, adolescents also engage more frequently in copulation with low-rank males and extra-group males (Perry and Manson 1995).

These observations might help understanding the pattern seen in Sumatran orangutans. The dominant flanged male often ignores the proceptive behavior of adolescent females (Utami Atmoko *et al*. 2009b).As the tenure of a dominant male is unlikely to reach 14 years, the age at which potential daughters become sexually active, I suggest that is unlikely that flanged males are not interested in young females to avoid inbreeding. Field observations suggest that nulliparous females require remarkably long periods (months, or even years) of sexual activity to get pregnant (Carel van Schaik, personal communication), indicating that their fertility might be initially much lower compared to older females. I suggest that the dominant male focuses his reproductive efforts on older females and only begins to show interest in nulliparous females when the chances of fertilization outweigh the effort. This strategy of the dominant male opens a window of opportunity to unflanged males. Even though most of the copulations with these females won't lead to conception, it is possible that every now and then a copulation between an unflanged males and a nulliparous female will result in a pregnancy.

5.2.3 Evidence for infanticide in Sumatran orangutans?

In primate species infanticide is a widespread behavior (van Schaik and Janson 2000, Watts 1989, Swedell and Tesfaye 2003, Struhsaker 1977, Clarke *et al*. 1994) and the potential benefits for the male perpetrators are of various nature (Ebensperger 1998).

The disappearance of at least four offspring in Suaq at the end of the 1990s has been observed (van Noordwijk and van Schaik 2005). As infant mortality for the first year in this species is generally very low at around 8% (Wich et al.2004), the disappearance of more than 30% of the infants in this population has been linked to human disturbance (logging and pet trade). In most cases involving the capturing of an offspring, the mothers are killed (Nijman 2005). However, in Suaq the mothers of the deceased offspring were still alive after the loss of their offspring. This suggests that infanticide could be a more reasonable explanation for the high mortality rates.

In orangutans, infanticide has never been documented (Beaudrot *et al*. 2009), even though there are reasons to believe that for orangutan males it would be relatively to kill unweaned offspring (van Schaik and Kappeler 1997). It is believed that Female orangutans of both species mate promiscuously and this behavior seems to induce paternity confusion, an effective counterstrategy to infanticide (Knott 2010, Stumpf *et al*. 2008).

Beaudrot *et al*. (2009) identified three conditions that need to be met in order to make infanticide an effective strategy for males: First, for males infanticide is only adaptive when the probability of siring the female's next offspring is higher than the probability of having sired the current offspring. Second, infanticide is only adaptive if females resume ovarian activity quickly after losing the current offspring. Third, males need to have access to the female after killing her current offspring.

The authors argue that at least two of these conditions are not met in Sumatran orangutans. In particular, based on a previous study of paternity for Ketambe (Utami *et al*. 2002), they argue that resident subordinate flanged males can be reproductively successful and thus would not benefit from infanticide. My results for Suaq seems to paint a different picture. Subordinate flanged males obtain virtually no copulations (Singleton and van Schaik 2002) and the results of my paternity analysis suggest that paternity is highly concentrated in the dominant male. I therefore argue that for both resident and transient subordinate flanged males, infanticide might be advantageous.

Beaudrot *et al.* (2009) also argue that female orangutans might not resume ovarian activity after the loss of an offspring. My maternity analysis confirms that Friska, who had lost a one year old offspring in 1998, had a new baby around 1999-2000. As energy availability has been linked to fertility in orangutans (Knott *et al.* 1999, but also Wich *et al.* 2006), it is possible that females in the energy-rich forests of Suaq might have the ability to give birth to a new offspring soon after losing one.

Thirdly, Beaudrot *et al.* (2009) argued that males in Sumatra should not kill infants because the access to the mother after the death is not guaranteed. This might be true most of the time but in the rare event of a male taking over a population, his future access to females is granted (Utami and Mitra Setia 1995).

The situation observed in Suaq at the end of the 1990s is peculiar. New, unknown flanged males were pushed into the study area by human activity and deposed the dominant male. In this situation it seems that it would be advantageous for the new males to kill the current offspring and might explain why infant mortality at the end of the 1997 and 1998 was so high.

In conclusion, a combination of high paternity concentration and monopolization potential, high infant mortality and dominance takeover by transient males could have led to infanticide in Suaq.

5.3 Limitations of this study and outlook

The lack of knowledge about male reproductive success does not lie in the lack of effort from the scientific community but are certainly linked to various aspects of orangutan's research. Firstly, the collection, storage and handling of non-invasive fecal samples in the Indonesian field sites represent an obstacle. Secondly, the effort required to obtain reliable genotypes from these samples is less time- and cost-effective compared to other types of samples (blood, tissue) and might discourage scientist from working with them. Thirdly, due to unpredictable ranging patterns and low densities, individuals are harder to find and to study over a long period. Lastly, orangutans have the slowest life history among non-human primates and females only produce offspring every 6-9 years (Galdikas and Ashbury 2012, Wich *et al*. 2004) making the gathering of sufficient data for paternity analysis very time consuming.

For my study, estimated year of birth, male developmental status at the time of conception and female reproductive history were generally very difficult to assess despite the longevity of the research projects for both populations. For this reason, in most cases reliable information for one or more attributes was missing and strongly affected my ability to determine the reproductive success of the two male morphs and how it is linked with the mother's reproductive history.

For what concerns the genetic analysis, I have used two powerful microsatellite datasets which allow to successfully discriminate between individuals and assign paternities at a satisfying confidence level (Fig 8-4- and 8-5). I have nonetheless encountered some limitations. As revealed from my analysis, intricate pedigree relationships were difficult to assess. The assessment of paternal half-sibs was very difficult, even in presence of the maternal genotypes. I suggest that the use of more markers might increase the power for relationship inference and more extensive sampling might provide better estimates of allele frequencies.

However, for the parentage analysis I have managed to achieve the 95% confidence level even in presence of mismatches and with an error rate set at 1%. This suggests that my dataset is powerful enough for likelihood analysis. In fact, even in presence of a mismatch, likely caused by genotyping mistake or a mutation (e.g. Nsubuga 2008), the remaining loci offer strong enough evidence in favor of a true parent-offspring relationship.

Despite the difficulties encountered, I think that the two datasets have the potential to unravel the mating systems for both species. For Suaq, field observation for the last 6 years suggest that a stable dominance hierarchy has been once again established. Paternity for the yet-to-be sampled 13 offspring born between 2003 and 2014 could reveal a similar picture to what was observed in the 1990s with Arno.

Similarly, in Tuanan, new samples have been collected and paternity for 17 more offspring born between 2003 and 2014 could in the future be assessed. The likelihood of having sampled the father of these recently born offspring is higher. In my data, most of the offspring were born before research started in and the father was likely gone by the time sample collection started in 2003. I have assigned paternity to five out of seven offspring analyzed who were born after the year 2000, and assigned 3 out of 10 analyzed who were born before the year 2000. Knowing that my markers are powerful enough to identify fathers, I suggest that in those cases the true fathers have not been sampled. Given the rather unstable social organization of flanged males in Borneo (Dunkel *et al* 2013), I suggest that the sampling of as many males as possible around time of conception is necessary for paternity analysis. The assignment of more paternities, in combination with the extensive behavioral, ecological and physiological data collected in the past ten years in Tuanan, might finally shed some light on this two species' mating systems.

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8 APPENDIX

8.1 Appendix: Materials and methods

Table 8-1: Microsatellite loci used in this study

MP	Marker ID	Repetition pattern	Sequence (5'→ 3')	Dye
HMP1	D1S550	Tetranucleotide	F: CCTGTTGCCACCTACAAAAG R: TAAGTTAGTTCAAATTCATCAGTGC	PET
	D2S1326	Tetranucleotide	F: AGACAGTCAAGAATAACTGCCC R: CTGTGGCTCAAAAGCTGAAT	NED
	D3S2459	Tetranucleotide	F: CTGGTTTGGGTCTGTTATGG R: AGGGACTTAGAAAGATAGCAGG	VIC
	D4S2408	Tetranucleotide	F: AATAAACTTCAACTTCAATTCATCC R: AGGTAAAGGCTCTTCTTGGC	FAM
	D5S1470	Tetranucleotide	F: CATGCACAGTGTGTTTACTGG R: TAGGATTTTACTATATTCCCCAGG	FAM
HMP2	D13S321	Tetranucleotide	F: TACCAACATGTTCATTGTAGATAGA R: CATACACCTGTGGACCCATC	PET
	D13S765	Tetranucleotide	F: TGTAACTTACTTCAAATGGCTCA R: TTGAAACTTACAGACAGCTTGC	NED
	D16S420	Dinucleotide	F: ATTTCCTGAGGTCTAAAGCACCC R: TTAGGCCCAGTCCACACTCAAG	VIC
	D2S141	Dinucleotide	F: ACTAATTACTACCCNCACTCCC R: TTTTCCAAACAGATACAGTGAACTT	FAM
	D5S1505	Tetranucleotide	F: TAAGTGCCAGAGTCTCCCAC R: TAAGGCATGTCTCGGAGCTA	FAM
	D6S501	Tetranucleotide	F: GCTGGAAACTGATAAGGGCT R: GCCACCCTGGCTAAGTTACT	PET
PMP1	D4S1627	Tetranucleotide	F: AGCATTAGCATTTGTCCTGG R: GACTAACCTGACTCCCCCTC	PET
	O4A5	Tetranucleotide	F: ATGGGCCCAGAAAACAACTCAGT R: AGATAAAGGAATGGATAGATGGACAGA	FAM
	O4A8	Tetranucleotide	F: CACAGGGTCCAAACTCAGATTATTG R: GTTTCCTTCCCCCTCATGTAGTTATCAA	NED
	O4B24	Tetranucleotide	F: TCTGAGGTACCCTGTAACAAAGAAA R: GAAATCCCAGTACCATATAAATGTCAT	FAM
	O4B5	Tetranucleotide	F: GAGCCCTGATTCGTTTTACTGG R: AGCAAAGGCAGAAAACTGTAATGA	VIC
	O4B6	Tetranucleotide	F: TGGAGCCTGAATATGTGACTGAAT R: AATGCCAGGATTTCCTTCTTTTT	FAM
	O4_6	Tetranucleotide	F: GGCAATGTAACATATCCCTCTGTGT R: AGCCATGGACCTTGTGAGAAAAG	PET
PMP2	D5S1457	Tetranucleotide	F: TAGGTTCTGGGCATGTCTGT R: TGCTTGGCACACTTCAGG	FAM
	O4A1	Tetranucleotide	F: CTCCCCTTCCTTCCTTTATTCAGTT R: CAACACTTGGCAGTCACAAATCAG	FAM
	O4B17	Tetranucleotide	F: GTACCGACGGTGCACGAACAATGTA R: AGCCTGGCTGAAAAGTGGAACTGAG	PET
	O4B3	Tetranucleotide	F: TTCCAGAAGGGGCGAGAAGTT R: GTTGGGACCAAACAGTTGTCAATAA	VIC
	O4C13	Tetranucleotide	F: CTGGGCACACTGTATATGGGGTAG R: GTTTGAGACCACTCATGATGCAAAGACC	FAM
	O4C9	Tetranucleotide	F: TGCAGGCCAGGGCTTCTTTCAA R: CAGTCTCCCCAGGACCCCTACACAG	FAM
PMP3	O4A7	Tetranucleotide	F: ACTGGCCCATTCAAAGTCTGTCATT R: ACTGGCCCATTCAAAGTCTGT	VIC
	O4B20	Tetranucleotide	F: CCTGCATTTTGTCACTCCCTCAACC R: CTGCCACACCTCCATGGACACAGAT	NED
	P_TETRA_Chr5	Tetranucleotide	F: CAGCAGCTCCTGAAATATCTGTCC R: GTTTGGGGTAGAGGAAAGCAGGTTGAT	PET
	P_TETRA_Chr7	Tetranucleotide	F: CATCTCTTTATGGCTGACTGTTGAT R: GTTTGGTCCAAGACAAATTTGTATGAGT	NED

8.2 *Appendix: Results*

8.2.1 DNA extraction

76 samples were extracted for a total of 95 extractions performed. DNA quantification was completed over 5 separate qPCR reactions. The correlation of determination R^2 for the five Standard curves always exceeded 0.95 and averaged 0.9882±0.0049, indicating that the estimates for the DNA concentration in the extracts are reliable.

Estimates revealed that concentrations of target DNA varied between 0 pg/ul and 3.3 ng/ul with an average of 151.28 pg/ul (table 8-2). When divided in the categories presented in Morin 2001, the distribution of DNA concentration appeared to be satisfying, with 40% of the samples reaching and exceeding 25 pg/ul (figure 8-1). 11 extracts appeared to contain no DNA but it is possible that a high concentration of PCR inhibitors prevented its amplification and thus its detection.

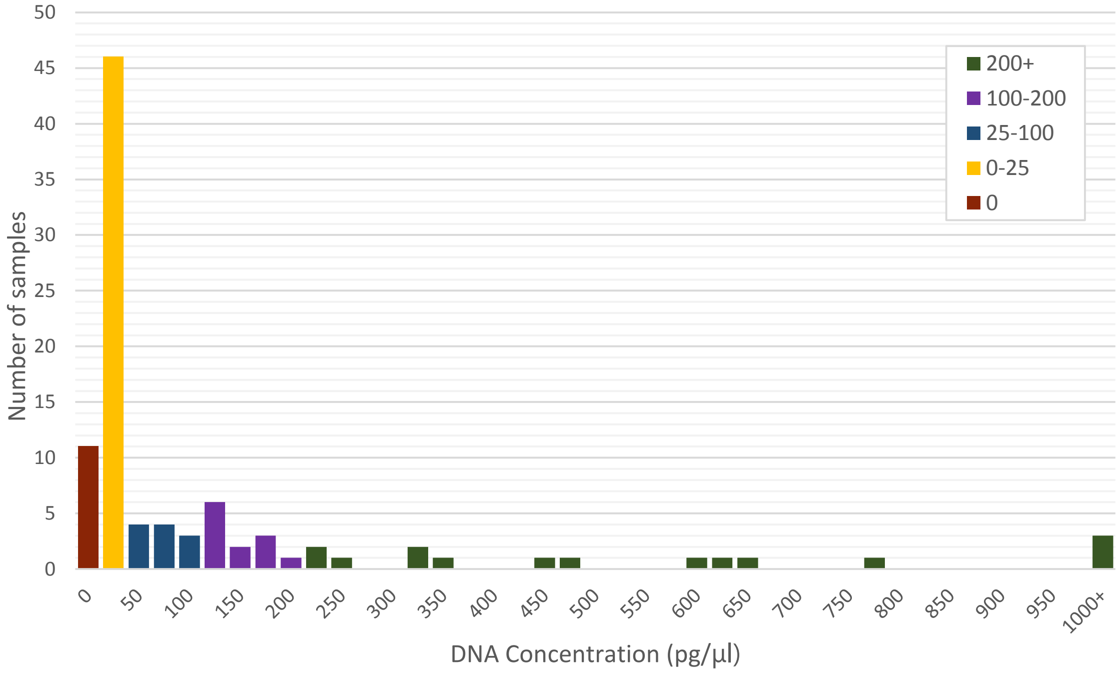

Figure 8-1: Distribution of DNA concentration in the extracts according to the 4 categories proposed by Morin *et al*. 2001

Samples with a concentration above 25pg/µl were used for the genotyping, even though observation suggested that also extracts with lower concentrations can produce satisfying results and an attempt at genotyping for the 6 HMP2 loci was made with several of these low-concentration extracts.

Table 8-2: DNA concentration, repetitions required according to Morin *et al*. (2001) and number of successfully typed loci for each extract.

Inventory Number	Mean DNA conc.	Repetitions	Loci typed
2077	1.494149	DNU	9
2099	1.204544	DNU	0
4288	12.96265	DNU	18
4293	26.95009	7	18
4314	1.756866	DNU	0
4326	67.07179	7	25
4351	5.724094	DNU	0
4353	0	DNU	0
4355	3.487272	DNU	0
4365	0.599858	DNU	0
4376	1.798685	DNU	0
4702	1.180709	DNU	0
4743	7.245081	DNU	0
5468	5.339406	DNU	0
5469	8.739554	DNU	3
5473	4.456352	DNU	0
5473	10.73956	DNU	18
5474	0	DNU	0
5474	0	DNU	0
5475	1.371463	DNU	0
5475	2.524452	DNU	0
5482	9.195361	DNU	14
5502	1.582077	DNU	0
5502	3.452386	DNU	0
5504	2.741074	DNU	0
5504	4.633104	DNU	0
5507	0	DNU	0
5507	3.912551	DNU	0
5509	8.745291	DNU	0
5510	0	DNU	0
5517	0	DNU	0
5517	4.221138	DNU	0
5521	0	DNU	0
5521	0	DNU	0
5521	1.884094	DNU	0
5522	0	DNU	0
5522	7.12949	DNU	0
5544	3306.575	2	27
5545	166.0757	4	0
5545	171.1551	4	10
5546	330.3779	2	27
5547	1056.113	2	9
5548	182.3777	4	10
5549	301.6974	2	10
5550	11.77066	DNU	0
5550	110.752	4	27
5554	324.3283	2	27

Table 8-2: Continued

5556	1.125215	DNU	0
5556	245.8101	2	24
5561	119.7313	4	25
5564	19.45194	DNU	27
5565	0	DNU	0
5565	2.88426	DNU	0
5565	8.958195	DNU	0
5566	26.12448	7	27
5567	10.30438	DNU	10
5569	4.853313	DNU	0
5569	99.22164	7	26
5575	6.722113	DNU	0
5575	29.92129	7	
5576	752.7161	2	0
5579	2.632999	DNU	
5581	66.39281	7	10
5583	6.400699	DNU	7
5584	9.563138	DNU	0
5584	15.51868	DNU	25
5585	5.604093	DNU	1
5586	117.1504	4	27
5590	473.5454	2	5
5591	151.0537	4	27
5594	145.8735	4	5
5602	624.6607	2	26
5604	640.0832	2	27
5610	2.426561	DNU	
5610	5.470359	DNU	
5611	78.10532	7	5
5615	1.227626	DNU	
5616	22.52458	DNU	27
5617	12.99845	DNU	5
5619	103.4046	4	5
5622	11.58196	DNU	25
5623	0	DNU	
5624	64.01858	7	5
5625	427.9674	2	5
5627	19.22052	DNU	18
5629	117.8612	4	16
5630	213.6555	2	16
5636	55.45231	7	9
5641	39.0487	7	26
5649	586.8771	2	26
5715	2282.97	2	0
5719	108.2326	4	5
5720	222.9017	2	27
5732	144.1035	4	26
5736	91.76767	7	9
Average	**151.27**		

8.2.2 Genotyping

After DNA quantification, 44 extracts were genotyped for the 6 HMP2 loci. Eleven extracts were duplicates from previously sampled individuals. 33 extracts were then genotyped for up to 22 additional loci. After encountering difficulties in the allele scoring process, I have discarded Locus D2S141 from the analysis.

Identity analysis showed that the 44 extracts exhibited 30 individual genotypes. Of these 30 unique genotypes, 9 matched with genotypes already present in the database that were produced by former members of the lab. This repetition of previously found genotypes nonetheless resulted to be particularly useful as it allowed to estimate the level of consistency in the allele scoring between the different people who worked on the creation of this dataset. The higher quality of several of the more recent samples I have extracted allowed me to identify some erroneous allele calls which could have heavily impacted the pedigree analysis. The final dataset for this population includes 57 unique genotypes and field observation and photos allowed to assign this genotypes to the corresponding individuals.

8.2.3 Error rates

Error rates were estimated comparing repeated genotypes and 4 mismatches were found among 310 allele comparisons, 2 of which were found at one locus in one of the comparisons. After examination it was obvious that for that particular locus the error was introduced in the allele calling process and had nothing to do with the quality of the extracts. The error rate was then estimated to be approximately 1%. For the Tuanan dataset, the error rate had been estimated to be 0.112% (Arora *et al*. 2012).

8.2.4 Evaluation of the genetic markers

8.2.4.1 Suaq

The Hardy-Weinberg test revealed that the loci D6S501, D4S1627, O4A7, PON_TETRA_Chr5 and PON_TETRA_Chr7 were not in Hardy-Weinberg equilibrium but after Bonferroni correction (new threshold for p-values= 0.001852), only locus D4S1627 appeared to be in disequilibrium and was discarded. Further testing for HWE was performed in GenAlex and resulted in the exclusion of two additional loci, O4A7 and PON_TETRA_Chr5.

Testing for Linkage Disequilibrium using Genepop showed that no genetic disequilibrium was found in between the remaining tested loci. A significance-threshold of 0.001852 for the p-values was used. Additionally, locus O4B20 was found to be monomorphic and was also removed from the set of genetic markers used in the downstream analysis. The resulting and final dataset was therefore reduced to 23 loci.

8.2.4.2 Tuanan

After testing for Hardy-Weinberg disequilibrium I have removed locus O4A8 from the dataset. Loci 04B2O and O4B3 resulted to be uninformative and were also excluded and not used for the rest of the analysis. The final dataset comprises 25 loci.

8.2.5 Investigation of descriptive genetic parameters and analytical power

In the final step before paternity analysis, I have performed a series of tests aimed at assessing the suitability of both my datasets for paternity analysis. Results for the number of allele per locus (N_A), expected (H_E) and observed (H_O) heterozygosity, Polymorphic Information Content (PIC) and probability of identity for both unrelated individuals (P_i) and siblings (P_{is}) are summarized in tables 8-2 and 8-3. Figure 8-2 and 8-3 show how P_i and P_{is} decrease by including more loci in the analysis

Table 8-2 Genetic parameters for the markers used for the Suaq population. N=Number of individuals genotyped, N_A= Number of alleles for the marker, H_o= Observed Heterozygosity, H_E= Expected Heterozygosity, PIC= Polymorphic Information Content, P_i= Probability of identity, P_{is} = Probability of identity (siblings), in italic: HMP2 loci

Locus	N	N_A	H_O	H_E	NE1P	NE2P	PIC	P_i	P_{is}
D1S550	56	6	0.839	0.775	0.632	0.454	0.729	0.092	0.389
D2S1326	56	3	0.339	0.374	0.931	0.808	0.339	0.428	0.672
D3S2459	57	4	0.772	0.708	0.722	0.553	0.649	0.142	0.434
D4S2408	53	5	0.698	0.667	0.768	0.613	0.596	0.18	0.465
D5S1470	55	5	0.745	0.692	0.733	0.563	0.635	0.149	0.445
D13S321	55	8	0.836	0.75	0.657	0.481	0.702	0.107	0.405
D13S765	55	6	0.6	0.652	0.768	0.615	0.582	0.19	0.474
D16S420	54	8	0.796	0.823	0.543	0.368	0.789	0.06	0.357
D5S1505	56	7	0.607	0.723	0.691	0.517	0.671	0.126	0.423
D6S501	56	4	0.5	0.632	0.798	0.646	0.561	0.205	0.488
O4A5	53	4	0.604	0.549	0.85	0.733	0.46	0.292	0.551
O4A8	55	3	0.218	0.242	0.971	0.886	0.218	0.599	0.78
O4B24	55	3	0.509	0.531	0.862	0.721	0.469	0.282	0.557
O4B5	54	6	0.796	0.745	0.672	0.497	0.694	0.113	0.409
O4B6	50	6	0.52	0.501	0.862	0.698	0.469	0.281	0.572
O4_6	55	4	0.582	0.675	0.755	0.596	0.608	0.171	0.458
D5S1457	56	6	0.768	0.711	0.701	0.521	0.666	0.126	0.429
O4A1	54	5	0.833	0.695	0.739	0.575	0.631	0.155	0.444
O4B17	54	6	0.741	0.688	0.743	0.579	0.624	0.159	0.449
O4B3	54	4	0.63	0.632	0.801	0.656	0.555	0.211	0.49
O4C13	49	4	0.755	0.626	0.805	0.668	0.541	0.223	0.496
O4C9	51	4	0.588	0.501	0.868	0.707	0.464	0.286	0.573
PON_7	42	13	0.786	0.835	0.494	0.324	0.81	0.046	0.349
Mean	**53.69**	**5.3913**	**0.6548**	**0.6403**	**1.1E-3**	**4.3E-06**	**0.5853**	**3.2E-16**	**1.8E-07**

Table 8-3 Genetic parameters for the markers used for the Tuanan population. N=Number of individuals genotyped, N_A= Number of alleles for the marker, H_o= Observed Heterozygosity, H_E= Expected Heterozygosity, PIC= Polymorphic Information Content, P_i= Probability of identity, P_{is} = Probability of identity (siblings), in italic: HMP2 loci

Locus	N	N_A	H_O	H_E	NE1P	NE2P	PIC	P_i	P_{is}
D1S550	40	7	0.65	0.709	0.71	0.541	0.65	0.14	0.435
D2S1326	45	3	0.556	0.521	0.867	0.731	0.456	0.294	0.566
D3S2459	43	6	0.837	0.812	0.57	0.392	0.774	0.068	0.366
D4S2408	39	5	0.744	0.653	0.759	0.583	0.604	0.166	0.469
D5S1470	44	4	0.477	0.499	0.874	0.736	0.441	0.309	0.581
D13S321	56	5	0.518	0.648	0.785	0.636	0.573	0.197	0.478
D13S765	55	5	0.6	0.658	0.77	0.617	0.586	0.187	0.471
D16S420	53	5	0.528	0.508	0.87	0.731	0.45	0.3	0.573
D2S141	54	8	0.833	0.769	0.634	0.457	0.724	0.095	0.393
D5S1505	53	8	0.755	0.77	0.618	0.438	0.732	0.087	0.39
D6S501	51	8	0.745	0.722	0.687	0.509	0.675	0.121	0.423
D4S1627	36	6	0.694	0.644	0.763	0.586	0.598	0.17	0.475
O4A5	38	6	0.658	0.676	0.737	0.559	0.628	0.15	0.454
O4B24	20	2	0.05	0.05	0.999	0.976	0.048	0.906	0.952
O4B5	41	5	0.659	0.619	0.791	0.626	0.564	0.199	0.494
O4B6	37	7	0.405	0.747	0.652	0.47	0.705	0.101	0.407
O4_6	42	3	0.738	0.618	0.813	0.674	0.535	0.227	0.501
D5S1457	42	7	0.857	0.785	0.618	0.44	0.74	0.086	0.384
O4A1	40	4	0.825	0.751	0.682	0.507	0.693	0.115	0.408
O4B17	37	7	0.73	0.802	0.581	0.402	0.763	0.072	0.373
O4C13	32	4	0.406	0.567	0.842	0.736	0.46	0.293	0.544
O4C9	39	4	0.564	0.647	0.78	0.627	0.574	0.195	0.479
O4A7	39	2	0.333	0.315	0.952	0.869	0.262	0.523	0.726
PON_TET	36	6	0.806	0.786	0.619	0.44	0.739	0.087	0.384
PON_TET	34	15	0.912	0.91	0.345	0.208	0.888	0.019	0.307
Mean	**41.8**	**5.68**	**0.635**	**0.647** **0.64844**	**2.5E-4**	**4E-07**	**0.594**	**6E-21**	**5.5E-09**

0.64844

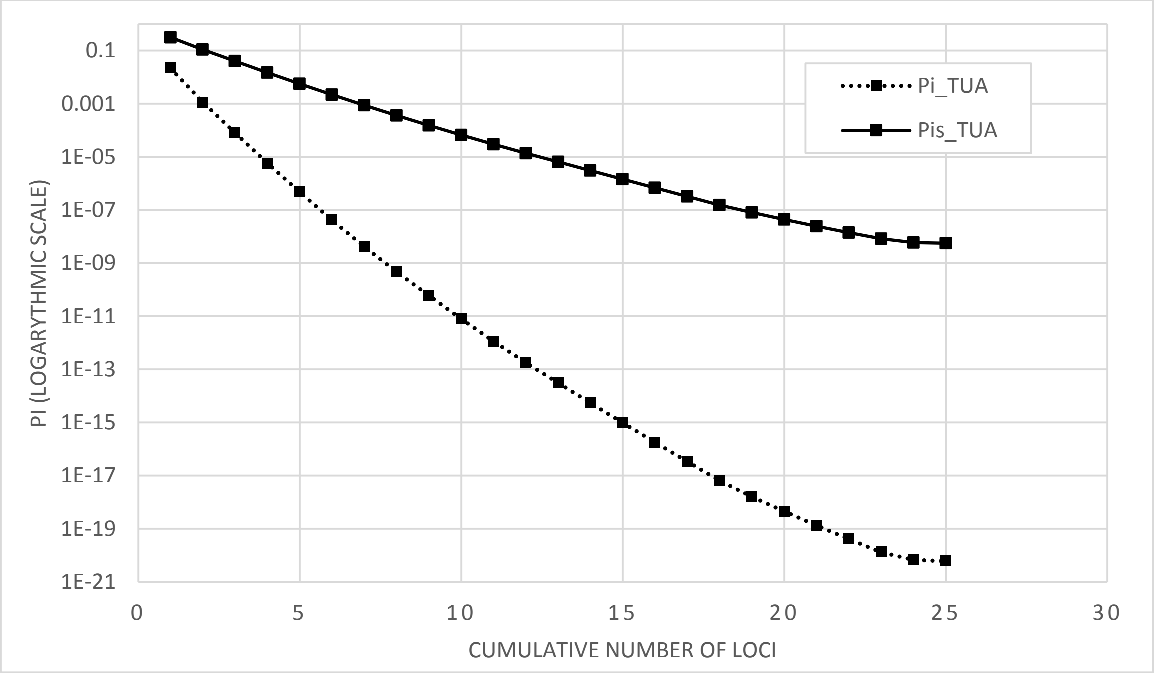

Figure 8-2: Cumulative probability of identity for Tuanan

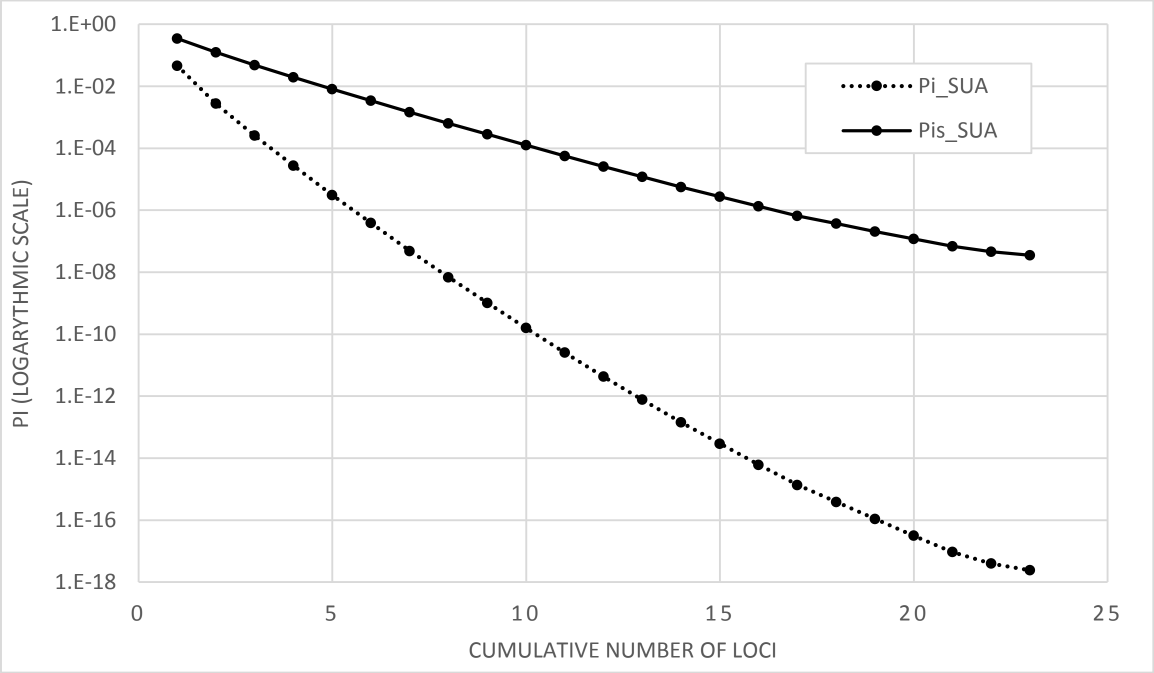

Figure 8-3: Cumulative probability of identity for Suaq

Figures 8-4 and 8-5 shows the results for the analysis of informativeness with KinInfor. These results suggest that for both populations, my dataset is powerful enough for the identification of parent offspring pairs, even in presence of full siblings. (74% of successfully discriminated pairs at 95% significance level for Suaq, 82% for Tuanan).

The results of this analysis also show that without a basic initial pedigree reconstruction, it would be difficult to identify half-sib pairs with necessary confidence. In fact, in approximately 30% of the cases, my dataset won't be strong enough to discriminate between half-sibs and unrelated individuals. This problem accentuates when other distantly related individuals are present in the population. For this reason I have decided that it will be necessary to initially identify parent offspring, the most easily identified relationship with my dataset, and then proceed in building an initial pedigree with these findings. The identification of half-sibship for pairs in which at least one of the four parents is known will be easier.

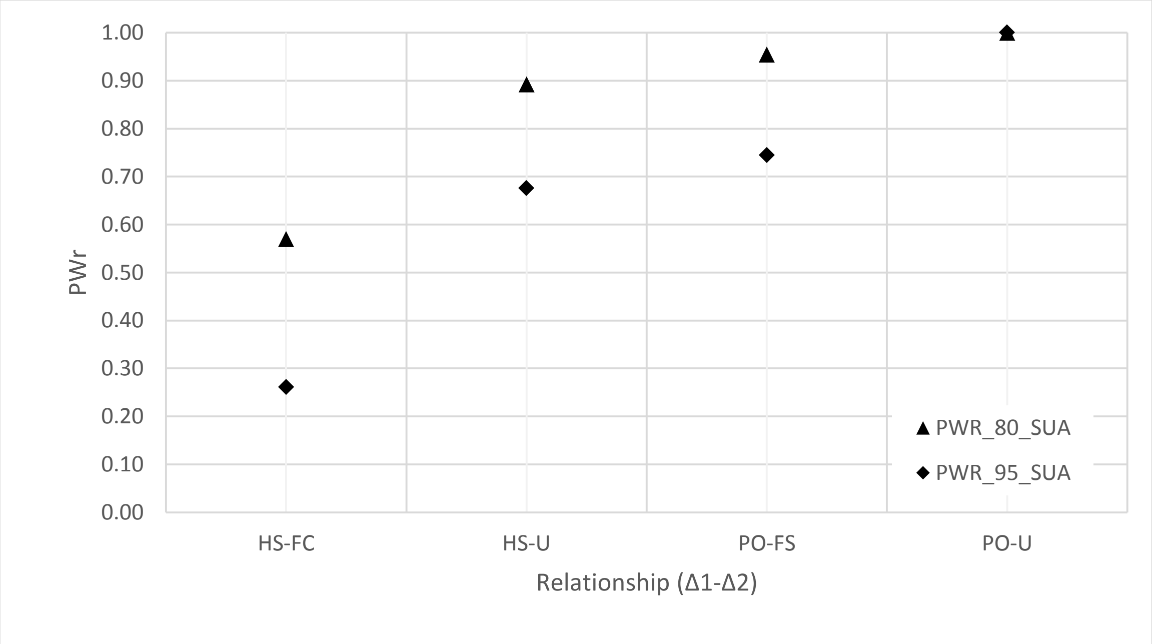

Figure 8-4: Estimates of PW$_R$ values for 4 hypotheses pairs for Suaq. HS=Half-siblings, FC=First cousins, U=Unrelated, PO=Parent-offspring, FS=Full siblings).

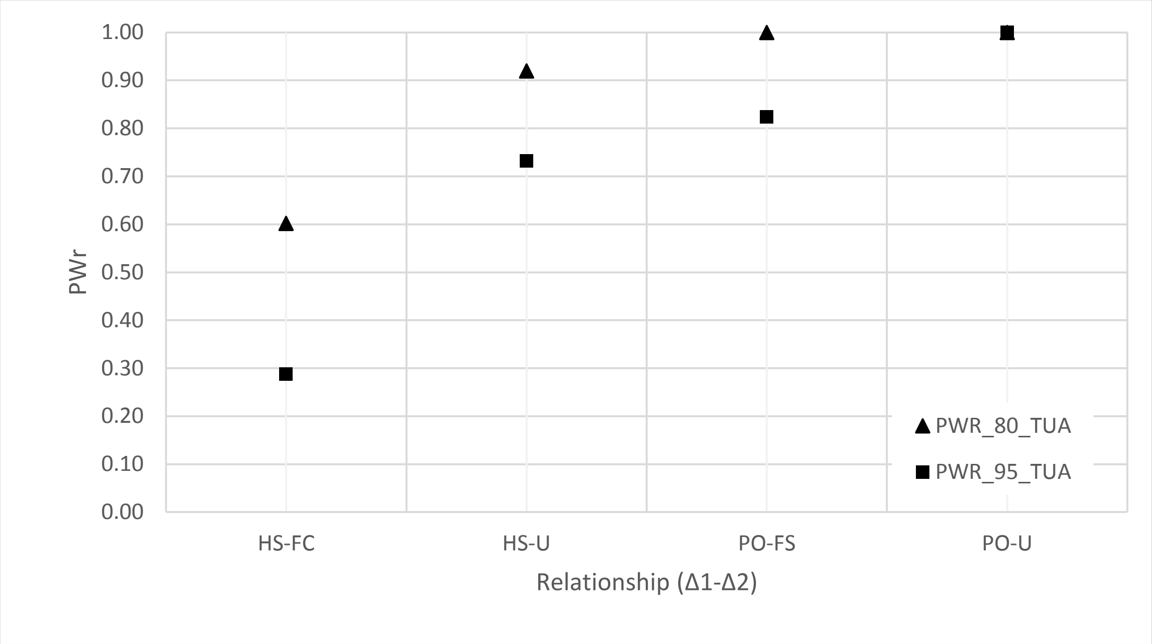

Figure 8-5: Estimates of PW_R values for 4 hypotheses pairs for Tuanan. HS=Half-siblings, FC=First cousins, U=Unrelated, PO=Parent-offspring, FS=Full siblings)

8.2.6 Parentage analysis: Parameters used

Table 8-4: Parameters used for the parentage analysis in Cervus

Analysis parameters in Cervus 3.0	Maternity		Paternity	
	Suaq	Tuanan	Suaq	Tuanan
Number of offspring	10000	-	10000	10000
Number of candidate mothers/fathers	45	-	100	100
Proportion of mothers/fathers sampled	0.5	-	0.25	0.25
Proportion of loci typed	0.94	-	0.94	0.755
Proportion of loci mistyped	0.01	-	0.01	0.01
Error rate	0.01	-	0.01	0.01
Confidence determined using	Delta	-	Delta	Delta
Relaxed confidence level	80%	-	80%	80%
Strict confidence level	95%	-	95%	95%

Table 8-5: Parameters used for the parentage analysis in COLONY

Analysis parameters in COLONY 2.0	Maternity		Paternity	
	Suaq	Tuanan	Suaq	Tuanan
Number of loci	23	25	23	26
Number of offspring in the sample	18	18	22	27
Outbreeding (0) or inbreeding (1) model	0	0	0	0
Number of male candidates	0	0	23	23
Number of female candidates	18	10	18	9
Number of known paternal sibships	0	0	0	0
Number of known maternal sibships	0	0	0	9
Number of offspring with excluded fathers	0	0	0	0
Number of offspring with excluded mothers	0	0	0	4
Male mating system	Polygamy	Polygamy	Polygamy	Polygamy
Female mating system	Polygamy	Polygamy	Polygamy	Polygamy
Number of Excluded Paternal Sibships	0	0	0	0
Number of Excluded Maternal Sibships	0	0	0	0
Seed for random number generator	1234	1234	1234	1234
Allele frequency	Update	Update	Update	Update
Sibship complexity prior	Yes	Yes	Yes	Yes
Length of run	Long	Long	Very Long	Long
Likelihood	High	Very High	Very High	Very High
Probability a mum is included in the female candidates	0.6	0.6	0.6	0.6
Probability a dad is included in the male candidates	-	-	0.25	0.25
Error rate	0.01	0.01	0.01	0.01

8.2.7 *Maternity: Suaq*

Maternity analysis in Cervus 3.0 and COLONY 2.0 revealed 17 mother-offspring pairs (Table 8-6).

Table 8-6: Results of maternity analysis performed with Cervus 3.0 and COLONY 2.0 (1Pne= First parent exclusion probability, 2Pne=Second parent exclusion probability, L_C= Number of loci compared, L_{MM}= Number of mismatches, Pair confidence= *=95%, +=80%)

Cervus								COLONY	
Offspring ID	1Pne	Mother ID	L_C	L_{MM}	Pair LOD	Pair ΔLOD	Pair confidence	Likelihood	Pair confidence
CHICK	9.46E-04	DODI	21	0	8.14	8.14	*	1.000	*
CHINDY	4.70E-03	CISSY	22	0	7.82	6.46	*	1.000	*
DIDDY	4.32E-04	DODI	19	0	8.62	7.24	*	1.000	*
DODI	2.26E-03	CHICK	21	0	8.14	8.14	*	1.000	*
ELLIE	8.32E-03	FRISKA	22	0	8.01	8.01	*	1.000	*
LENA	5.97E-04	DIANA	23	1[1]	5.25	5.25	*	1.000	*
LILLY	1.51E-03	LISA	23	0	4.74	4.70	*	1.000	*
LISA	5.98E-03	CISSY	22	0	6.35	2.65	*	1.000	*
NIBLA	5.04E-04	INTAY	23	0	9.52	9.52	*	1.000	*
RAFFI	8.61E-04	HALTE	20	0	7.16	5.80	*	1.000	*
ROBBI	2.27E-04	LENA	23	0	13.0	10.6	*	1.000	*
SHERA	1.55E-03	CHICK	23	0	5.26	5.26	*	1.000	*
HERDY	1.42E-03	FRISKA	22	0	6.62	6.62	*	1.000	*
TEDI	2.12E-04	HALTE	22	1[2]	4.74	3.23	*	1.000	*
TINA	5.11E-03	RAFFI	20	0	5.81	5.81	*	1.000	*
UNO	2.13E-03	BIB	21	0	9.56	9.56	*	1.000	*
HALTE	3.32E-03	FRISKA	22	0	4.58	4.58	*	1.000	*
FREDDY	2.92E-04	FRISKA	22	0	6.84	1.88	+ [3]	1.000	*

[1, 2] Mismatch likely caused by genotyping error

[3] Requirements for significance at strict confidence level were not met due to the presence of a female relative, probably on the paternal side, in the pool of candidate mothers (see Marshall *et al*. 1998, Kalinowsky 2007). Field observations allowed to confirm this relationship

8.2.8 *Paternity concentration: Suaq*

Table 8-8: Results of the reproductive skew analysis for Suaq with Skew Calculator 2013 for 8 estimators (Nonacs 2003).

Number of males	Number of successful males	S	Sc	S3	Q	λ	Iδ	Ip	*B*	P level
44	8	0.8317	0.2386	0.9009	0.0884	0.3194	4.8	0.552	0.0464	0.048

8.2.9 Maternity: Tuanan

Maternity analysis for post-dispersal individuals with Cervus 3.0 was already completed by former lab members (Arora *et al*. 2012). I performed a maternity analysis including juvenile individuals in COLONY and identified 15 mother-offspring pair (Table 8-7).

Table 8-7: results of the maternity analysis performed in COLONY 2.0 for Tuanan.

Offspring ID	Mother ID	Confidence level	Significance (*) = 0.95 (+) = 0.80
CIKIPOS	WILMA	1.000	*
DESY	INUL	1.000	*
FRODO	WILMA	1.000	*
IDO	INUL	1.000	*
MILO	MINDY	1.000	*
PUMUCKL	PINKY	1.000	*
STREISEL	SIDONY	0.999	*
SUSI	SUMI	0.977	*
JIP	JUNI	0.998	*
MINDY	JINAK	0.997	*
JERY	JINAK	0.988	*
KONDOR	KERRY	0.989	*
KERRY	JINAK	0.999	*
LOLO	SIDONY	0.985	*
JUNI	JINAK	0.988	*
TIKUS	TALIA	0.982	*