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The Genetic Consequences Of Female Mate Choice In Black Horned Capuchin Monkeys *(Sapajus nigritus)*

Abstract

Previous studies have elucidated female sexual behaviour in black horned capuchin monkeys (Sapajus nigritus). Based on proceptive behaviours, females have a clear alpha male preference. This study aims to understand the genetic consequences of this female preference through the use of microsatellite analysis on 16 loci across 70 individuals from three study groups from the Iguazú National Park, Argentina. This study has five objectives: (1) to determine whether rank can predict paternity, (2) whether a PoA determined expected success is statistically similar to observed success, (3) whether females are confusing paternity enough to prevent infanticide, (4) the utility from using microsatellite markers from a species from a different family, and (5) the utility of using DNA samples that were 5 to 12 years old. It was found that rank predicted paternity, and expected success was statistically similar to observed success. Added to this the alpha males (7 of 12 assigned offspring) were the most successful sires in the group, followed by gammas (3/12) and then deltas (2/12). Females were not confusing paternity enough to prevent infanticide. 16 of the 18 markers successfully amplified, although one was monomorphic, and eight of the remaining 15 loci, had high null allele frequencies. Old DNA samples worked best when a combination of samples and more than four repeats were performed.

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Contents

Cycles Spot 2011	Date	Sub	Number of proceptive females	TRU	RIN		
1	29/07/2011	CLA	2	1	1		
2	31/07/2011	SPO	2	1	1		
				0.5	0.5	1	**Exp (Suc)**
				0.5	0.5	1	PROP ES

Cycles Spot 2012	Date	Sub	Number of proceptive females	TRU	RIN	TET		
	08/06/2012	JOS	1	1	0	0		
	01/07/2012	JOS	1	1	0	0		
	09/07/2012	DAN	1	1	0	0		
				3	**0**	**0**	**3**	**Exp (Suc)**
				1	0	0	1	PROP ES

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Abbreviations

*: Strict Confidence

-: Most Likely Father (Parent) Not Assigned

+: Relaxed Confidence (80%)

Bp: Base Pairs

CI: Confidence Intervals

Dp: Decimal Places

EPCs: Extra-Pair Copulations

EPMs: Extra-Pair Mates

ES: Expected Success

F: Forward (Primer)

FL: Fluorescent (Fluoro-) Label

GLMM: Generalized Linear Mixed Model

H_0: Null Hypothesis

IBIs: Inter-Birth Intervals

LOD: Log Of The Overall Likelihood Ratio

MMS: Maya Marriott-Semper (Principal Investigator)

NAF: Null Allele Frequency

NC: Negative Control

OS: Observed Success

PCR: Polymerase Chain Reaction

PIC: Polymorphic Information Content

PoA: Priority Of Access Model

POP: Peri-Ovulatory Periods

PSC: Phylogenetic Species Concept

R: Reverse (Primer)

RFU: Relative Fluorescence Units

RS: Reproductive Success

SSI: Sexually Selected Infanticide

Δ: DELTA

Subject Information

Table 1. Showing names and ID code/number used to refer to subjects during the study

ID Code and Number	ID name
ALE36	Alela
ANI37	Anibal
AST1	Astor
BAR38	Bardo
BEA39	Beau
BIA2	Bianca
BOR3	Borat
CAB40	Cambai
CAM41	Camilo
CLA4	Clara
COR5	Cordelia
DAL42	Dali
DAN6	Daniela
DAT43	Dante
DAV44	David
EDU7	Eduardo
ELE8	Elena
ELO45	Eloy
ERN9	Ernesto
ESB46	Esteban
EST10	Estela
EVA11	Eva
FEN47	Feny
FRA48	Francisco
GUE49	Guenon
GUI50	Guiditta
HOR51	Horacio
HUR52	Hurlio
ILA53	Ilaria
ING12	Ingrid
ISI54	Isidro
JAC55	Jack
JOS13	Josefa
JC56	Juan Carlos
LEN57	Lenin
LIL14	Liliana
MGO61	Magoo
MAR16	Marcelo
MAT58	Matilda
MAV59	Maverick
MAW17	Maw

MAY18	Maya
MER60	Merlot
MIL62	Milton
MOJ63	Mojo
MOR64	Mora
MUR65	Murtang
OCT20	Octavio
OFE21	Ofelia
OMA22	Omar
PAC24	Pacino
PIE25	Pietro
RIC66	Ricky
RIN26	Ringo
RIT67	Rita
ROB68	Roberto
SEA69	Sean
SER27	Sergio
SRP29	Serpico
SIM70	Simon
SOL28	Sol
SPC71	Spartacus
SPO72	Spot
SYR73	Syrah
TET74	Tetro
THE30	Thelma
TTO32	Tito
TRU31	Trucho
VEL33	Velmiro
YOL35	Yoli
ZIS75	Zissou
ALS76	Alessandro
CHA77	Chango
HOM78	Homero
JES79	Jesus
JUS80	Justo
CAR81	Carmela
CHI82	Chicca
Note individuals in red had no faecal or hair samples collected and therefore no DNA extracted was extracted from them.	

Introduction

Literature Review

Introduction to Literature Review

Evolutionary theory dictates all assumptions and explanations in biological research. Therefore, Darwin's (1859, 1871) theories on natural and sexual selection, are basic tenets that are central to the study of animals. Sexual behaviour and resulting reproductive success from sexual encounters is a complex matter to study because sexual encounters involve two individuals of different sexes with different evolved strategies for maximising individual reproductive success irrespective of whether it benefits their partners'. This leads to conflicts between the sexes (intersexual conflict) and the evolution of strategies and counterstrategies by each sex (Bateman, 1948; Mayr, 1972; Trivers, 1972; Goossens *et al.*, 1998; Cohas *et al.*, 2006; Clutton-Brock, 2007; Reichard, 2009). In addition, individuals of one sex often compete (intrasexual selection) over sexual access to individuals of the other sex. This results in a reproductive skew and leads to the evolution of more drastic strategies to ensure reproductive success (Fleagle, 1988; Van Schaik, 2000; Gerald, 2001; Pradhan *et al.*, 2006; Knott and Kahlenberg, 2007; Bergman, Ho and Beehner, 2009; Lukas and Huchard, 2014; Strier, 2016).

Whether a trait evolves due to natural or sexual selection can be difficult to determine. Mayr (1972) argues that often sexually dimorphic traits like body and canine size in many primates, are solely thought to be the result of sexual selection. However, these traits could also increase survival, and allow the different sexes and demographic groups to occupy slightly different niches. This may be the case in male orangutans (*Pongo pygmaeus, Pongo abelii*), where both flanged and unflanged males exist who both seem to be reproductively successful (Knott and Kahlenberg, 2007). Unflanged males are males at reproductive age who have not developed of some male secondary sexual characteristics like increased body size and flanges. Males can transition between flanged and

unflanged states. Due to flanged males' larger size and decreased arboreality, flanged males may be able to exploit a different niche from the rest of its conspecifics, leading to decreased competition and perhaps survival (Galdikas, 1995; Knott and Kahlenberg, 2007; Redmond, 2010). Another possibility is that the flanged state and its corresponding dimorphism may be costly to survival and may be a solely sexually selected trait, which may be evidenced by the seemingly high cost of flanges. Despite the ambiguity of whether flanges are naturally (as well as sexually) selected, it is clear that both are continuous processes which work together to maximise survival and reproductive success.

Sexual Selection: Mating Strategies, Parental Investment

Males and females have different mating strategies that lead to optimum success (Bateman, 1948; Mayr, 1972; Trivers, 1972). In Bateman's (1948) study on fruit flies (*Drosophila),* he found that males could increase their reproductive success by mating promiscuously, while promiscuous mating did not increase a female's reproductive success. This is because, the more females a male mates with, the more possible successful inseminations a male can have. Once a female is inseminated, there is only one reproductive outcome. Therefore, in the same space of time a male can produce many more offspring than a female. Since Bateman's (1948) paper, however, it has been found that females also benefit from promiscuous mating (Trivers, 1972; Goossens *et al.*, 1998; Cohas *et al.*, 2006; Clutton-Brock, 2007; Reichard, 2009). This is because although polyandrous mating does not affect the number of offspring a female produces it can increase offspring genetic quality (for other benefits see below). For example, in the monogamous alpine marmots (*Marmota marmota)* (Cohas *et al.*, 2006) found that female extra-pair copulations (EPCs) were more common when extra-pair mates (EPMs) were more heterozygous or less closely related to the females than their monogamous mates. Through EPCs females are able to choose males with better genetic quality while still having mates to help with parenting. This strategy, while extremely beneficial to the

female, is extremely detrimental to her mate, who wrongly invests in another male's offspring (Trivers, 1972; Cohas *et al.*, 2006). Therefore, promiscuous mating can enhance reproductive success for both sexes. However, the promiscuous mating of one sex often impacts reproductive success of the other. For females, promiscuous mating often limits paternal care due to lower levels of paternal confidence. For males, a female mating promiscuously decreases his chance to sire offspring.

Another aspect of intersexual conflict is based on the different levels of parental investment between the sexes, which leads to different optimum strategies (Bateman, 1948; Mayr, 1972; Trivers, 1972). Parental investment relates to any contribution of a parent towards an offspring's survival but which also impedes the parent's ability to contribute to another offspring (Trivers, 1972). Parental investment, among other behaviours, involves gamete production and investing in offspring survival and growth. Parents must make a trade-off between offspring quantity and quality. Across the animal kingdom, the sex that invests more in usually the female, and paternal investment varies immensely. The amount of parental investment a male offers, normally depends on his paternity certainty . Paternal investment is also affected by mating systems, with more paternal investment occurring in monogamous, as paternity is more certain rather than promiscuous mating systems, where paternity is less certain (Trivers, 1972; Møller and Birkhead, 1993; Kappeler and van Schaik, 2002; Cohas *et al.*, 2006). Furthermore, paternal investment is higher in species where offspring survival greatly increases with investment from multiple individuals (Trivers, 1972). For males then an optimum strategy involves minimal parental investment and mating with as many females as possible, who are not mating with any other males (polygyny). A female optimum strategy could involve mating with one male who provides substantial parental investment (monogamy).

As a result of increased parental investment females tend to be the choosier sex, and are more discriminate in choosing their sexual partners. This lead to both inter-sexual and intrasexual selection. Intrasexual selection is the competition within a sex over access to mates (normally males) or resources with increase reproductive success (usually females) (Darwin, 1871; Bateman, 1948;

Mayr, 1972; Trivers, 1972; Gowaty and Hubbell, 2005, 2005; Clutton-Brock, 2007). This competition over access varies. In primates alone, monogamous, polygynous, polyandrous and polygynandrous mating systems are all seen (Janson and Van Schaik, 2000; Van Schaik, 2000; Kappeler and van Schaik, 2002; Maestripieri and Roney, 2005; Pradhan *et al.*, 2006; Redmond, 2010; Lukas and Huchard, 2014; Palombit, 2015; Strier, 2016). The amount of male-male competition varies depending on the mating system. In monogamous systems, access to females is much less constrained as the majority of males will be able to mate with a female and form a monogamous pair (Trivers, 1972; Kappeler and van Schaik, 2002; Strier, 2016).. Monogamy is related with a low level of male intrasexual competition. In polygynous mating systems, male forms a sexual (and social) grouping with multiple females. This increases male-male competition, as many males will not get a chance to mate with females unless they gain access to a harem. Polygyny then leads to high rates of intrasexual sexual selection especially in the form of agonism. In polyandrous and polygynandrous mating systems as females mate promiscuously, male-male competition normally occurs in the post-copulatory contexts, like sperm competition. When females mate promiscuously male intrasexual selection rates are intermediate (Trivers, 1972; Kappeler and van Schaik, 2002; Strier, 2016).

In males intrasexual selection often manifests in the form of agonism. This can lead to the evolution of weapons, like horns in the stag, which are absent or less pronounced in the female (Darwin, 1871). In primates this is often seen with canine size dimorphism (Fleagle, 1988). Another aspect of male morphology that is linked to both inter- and intra- sexual selection is the evolution of ornaments. Examples of ornaments include the red chest patch of geladas *(Theropithecus gelada)* (Bergman, Ho and Beehner, 2009) and the blue scrotum of vervets (*Cercopithecus aethiops sabaeus)* (Gerald, 2001). The intensity of colour, which differ among individuals, seems to signal to conspecifics social status It also allows females to choose the ideal mate and males to be able to assess the strength of their competitors.

The Male Perspective: Infanticide And The Priority Of Access Model

One behaviour that has been sexually selected in males as a result of both intra- and inter- sexual conflict is infanticide. Infanticide is the fatally wounding an infant or young offspring by a conspecific, is seen throughout the animal kingdom in a variety of forms (Hrdy and Hausfater, 1984), in particular males have often been observed to kill unrelated infants. Many hypotheses have been put forward to explain this, including social pathology, where infanticide is thought to be a maladaptive behavioural response to human disturbance. Another theories include: the cannibalism of an infant for resources, resource competition, reduction of future rivals and a by-product of male intrasexual competition (Hrdy and Hausfater, 1984; Van Schaik, 2000; Feh and Munkhtuya, 2008; Palombit, 2015). The most supported hypotheses is sexually selected infanticide (SSI) (Hrdy, 1974; Hrdy and Hausfater, 1984; Pusey and Packer, 1994; Janson and Van Schaik, 2000; Van Schaik, 2000; Van Schaik, Hodges and Nunn, 2000; Bellemain, Swenson and Taberlet, 2006; Feh and Munkhtuya, 2008; Boyko and Marshall, 2009; Clarke, Pradhan and Van Schaik, 2009; Zhao, Borries and Pan, 2011; Lukas and Huchard, 2014; Palombit, 2015). For SSI to be adaptive certain criteria must be met (Hrdy, 1974; Hrdy and Hausfater, 1984; Van Schaik, 2000; Bellemain *et al.*, 2006; Bellemain, Swenson and Taberlet, 2006; Feh and Munkhtuya, 2008; Clarke, Pradhan and Van Schaik, 2009; Palombit, 2015). Firstly, the male must be highly unlikely to be the father of the infant. Secondly, killing the infant must reduce the time it takes the female (infant's mother) to return to oestrous. Finally, the infanticidal male must be likely to sire the female's next offspring.

SSI was originally hypothesized to be most prevalent in non-seasonally breeding species with polygynous mating systems and short alpha male tenure (Hrdy and Hausfater, 1984). However, infanticide, which has a low incidence rate due to effective female counterstrategies, is seen across primate species (Van Schaik, 2000; Palombit, 2015). For example, in the seasonally breeding ring-tailed lemurs (*Lemur catta*), infanticide and female counterstrategies have been observed (Pereira and Weiss, 1991; Palombit, 2015). During the first 5 months of an offspring's life, mothers were

more agonistic towards males that could not have sired their infants, than in other reproductive phases (Pereira and Weiss, 1991). Infanticide in seasonally breeding species can still be beneficial to males in seasonally breeding species. This is because infanticide may improve future female reproductive output (Van Schaik, 2000; Palombit, 2015). For example, when Inter-birth Intervals are long and cause females to miss a breeding season, infanticide is an adaptive male strategy (Van Schaik, 2000; Palombit, 2015). For example, SSI has been observed in the seasonally breeding black horned capuchins (Iguazú, Argentina population) (Ramírez-Llorens *et al.*, 2008). In this population, females with surviving offspring tend to reproduce approximately every two years (Di Bitetti and Janson, 2001a; Ramírez-Llorens *et al.*, 2008; Janson, Baldovino and Bitetti, 2012). This is despite it being possible for females to mate in successive years as seen in a few cases in years with high provisioning (Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012). Furthermore, when infants did not survive past 8 months, females were likely to have shorter IBIs which allowed females to reproduce in the next breeding season (Ramírez-Llorens *et al.*, 2008; Janson, Baldovino and Bitetti, 2012).

Another aspect that affects male sexual behaviour and reproductive success is dominance hierarchies. Dominance hierarchies are seen across the primate order. All primates have dominance hierarchies within and between the sexes, although the amount despotism varies across species and populations (Strier, 2016). This hierarchy can influence male mating success either via coercion, mate guarding and harem keeping abilities or due to female preference of alpha males (Clarke, Pradhan and Van Schaik, 2009; Strier, 2016). Coercion in sexual behaviour relates to a male's ability to physically attack or threaten a female into copulating with him (Muller, Kahlenberg and Wrangham, 2009). Mate guarding, related to coercion, is a male's ability to prevent females from mating with other males (Muller, Kahlenberg and Wrangham, 2009). In addition, SSI is an especially adaptive strategy when there is a high reproductive skew, because males are able to greatly increase their limited reproductive window with the reduction of IBIs. Coercion is mainly seen in catarrhines. Furthermore, the adaptiveness of coercion will vary as females evolve counterstrategies to lessen its

effect (Clarke, Pradhan and Van Schaik, 2009). However the predictions of a male's ability to monopolise females to enhance his reproductive success, has led to the priority of access model (Pope, 1990; De Ruiter, Hooff and Scheffrahn, 1994; Altmann *et al.*, 1996; Gerloff *et al.*, 1999; Clarke, Pradhan and Van Schaik, 2009; Dubuc *et al.*, 2011; Van Belle *et al.*, 2014). The Priority of Access (PoA) model explains the male reproductive skew. It assumes that the alpha male's reproductive success is linked to this ability to monopolise females in the group which is done via mate guarding. In addition, it predicts that females are harder to monopolize when their oestrous periods overlap. This is because the model assumes that males can only successfully monopolize one female at a time. The model also predicts that when females' cycles are synchronised, beta and lower ranking males will also get breeding opportunities. Therefore, this model allows for the test of male reproductive skew, predicting that the proportion of reproductive opportunities a male has is directly related to both his rank and the number of available females (Pope, 1990; De Ruiter, Hooff and Scheffrahn, 1994; Altmann *et al.*, 1996; Gerloff *et al.*, 1999; Clarke, Pradhan and Van Schaik, 2009; Dubuc *et al.*, 2011; Van Belle *et al.*, 2014).

The Female Perspective: Female Mate Choice, Constraints And Counterstrategies

One problem, however, with the PoA model is that it will be less likely to apply in cases in which female mate choice affects mating success (Dubuc *et al.*, 2011). Female mate choice is an important factor which often determines male reproductive success. For example, in chimpanzees (*Pan troglodytes schweinfurthii*) alpha males had the most reproductive success, siring over a third of the offspring. However, alpha males were also unable to monopolize females enough to prevent females from mating with other males (Constable *et al.*, 2001). Therefore, female mate choice may be a stronger predictor for male reproductive success. Females can choose mates based on their genetic quality, protective abilities, for access to resources and paternal care. Alphas often control food resources, thereby females may mate with these males to gain increased access or tolerance in

feeding areas. In addition, alphas can offer protection to females and their infants, and in groups with high infanticide risk, males may protect their own offspring from infanticidal males (Fossey, 2000; Zhao, Borries and Pan, 2011; Van Belle *et al.*, 2014; Palombit, 2015). In some species alpha males may also have superior genetic quality, which is signalled to females (Bergman, Ho and Beehner, 2009). In addition, PoA does not take into account inbreeding avoidance strategies, whereby individuals avoid mating with closely related group mates. For example, it is often observed that when females reach maturity in groups where the alpha male who has had a long tenure and is most likely the female's father, these females and alphas will not mate (Pereira and Weiss, 1991; Pusey and Packer, 1994; Escobar-Páramo, 1999; Constable *et al.*, 2001; Di Bitetti and Janson, 2001a; Bellemain *et al.*, 2006; Cohas *et al.*, 2006; Muniz *et al.*, 2010; Dubuc *et al.*, 2011; Van Belle, Estrada and Di Fiore, 2014).

Male mammals, including primates seem unable to recognise their offspring based on their phenotypic characteristics. They instead seem to rely on mating histories and proximity to the mother prior to the infant's birth (Hrdy and Hausfater, 1984; Van Schaik, 2000; Palombit, 2015). Because of this, female counterstrategies to SSI often involve changing mating behaviour. In primates it is more common to see females being proceptive/receptive during pregnancy and/or lactation, especially in the presence of potentially infanticidal males (Hrdy, 1974; Clarke, Pradhan and Van Schaik, 2009; Izar *et al.*, 2009; Palombit, 2015). Females also have concealed and unpredictable ovulations (Van Schaik, Hodges and Nunn, 2000; Clarke, Pradhan and Van Schaik, 2009; Palombit, 2015). Males are privy to approximate but not exact timings of ovulation, and thus approximate likelihood of conception (called the approximate window). This approximate window, which lasts longer than the sperm can survive in a female's reproductive tract, can allow females to bias paternity certainty (Van Schaik, Hodges and Nunn, 2000).

Perhaps the most common female counterstrategy to infanticide is paternity confusion via female promiscuous mating (Hrdy, 1974; Hrdy and Hausfater, 1984; Pope, 1990; De Ruiter, Hooff and Scheffrahn, 1994; van Schaik and Janson, 2000; Wolff and Macdonald, 2004; Bellemain *et al.*, 2006;

Bellemain, Swenson and Taberlet, 2006; Feh and Munkhtuya, 2008; Ramírez-Llorens *et al.*, 2008; Boyko and Marshall, 2009; Clarke, Pradhan and Van Schaik, 2009). The cost of this counterstrategy for females is that it reduces direct female choice, as the female cannot direct all her mating effort towards her preferred male. By mating promiscuously with all the males present to decrease infanticide risk, direct female choice is eroded. This may in some cases lead to cryptic female choice. Through cryptic female choice, females can bias the paternity of their offspring while mating promiscuously (Maestripieri and Roney, 2005). This strategy would also benefit fathers of vulnerable infants. Furthermore, these fathers may even facilitate paternity confusion by allowing males to mate with females during less fertile periods of the oestrous cycle.

Aside from promiscuous mating, females employ other counterstrategies. Females with vulnerable young are also seen to be aggressive and/or avoid infanticidal males, emigrate from the group after takeover and wean infants more quickly (Pereira and Weiss, 1991; Van Schaik, 2000; Ramírez-Llorens *et al.*, 2008; Clarke, Pradhan and Van Schaik, 2009; Zhao, Borries and Pan, 2011; Janson, Baldovino and Bitetti, 2012; Palombit, 2015). Females with vulnerable infants will also protect infants. Group females may even band to together to protect vulnerable infants and more successfully drive off attacks (Palombit, 2015). In addition, probable sires many assist mothers in protecting their vulnerable infants (Palombit, 2015).

The length of an alpha male's tenure is thought to affect female promiscuity (Clarke, Pradhan and Van Schaik, 2009). In species with long alpha tenure (relative to IBI), promiscuity is less than in species with short alpha tenures. This is because: (1) females tend to prefer alphas (see below), (2) long alpha tenures lead to more stable group composition which reduces the risk of infanticide and (3) alphas are more likely to invest in offspring that they have a higher certainty for. This means that in groups with relatively long alpha tenures, during years of stability it would benefit females most to mate less promiscuously and focus mating on the alpha to ensure paternal investment and protection. Towards the end of these tenures, it would then benefit females to adopt a more

promiscuous mating strategy to protect their infants from infanticide in the case of an internal takeover (Clarke, Pradhan and Van Schaik, 2009). These are thought to be behaviours related to present infanticide risk and infanticide avoidance. In addition this lessening of promiscuity may help to explain why infanticide is a common strategy used by new alphas when they takeover a group (Hrdy, 1974; Hrdy and Hausfater, 1984; Van Schaik, 2000; Ramírez-Llorens *et al.*, 2008; Lukas and Huchard, 2014; Strier, 2016).

Female catarrhine primates, in contrast to other primates, tend to be subjected to more coercion and mate guarding by males (Van Schaik, Hodges and Nunn, 2000). This means that catarrhine males are better able to monopolise female mating access, erode direct female mate choice and reduce female promiscuity. Catarrhine primates also have longer follicular phases of oestrous cycles which may be a response to this increased male coercion (Van Schaik, Hodges and Nunn, 2000). Increased male coercion may have led to evolution of exaggerated sexual swelling and copulation calls seen across many catarrhine genera like baboons (*Papio* spp.), macaques (*Macaca* spp.) and bonobos and chimpanzees (*Pan* spp.) (Hamilton III and Arrowood, 1978; Nunn, 1999; Maestripieri and Roney, 2005; Pradhan *et al.*, 2006; Townsend, Deschner and Zuberbühler, 2008; Clarke, Pradhan and Van Schaik, 2009; Campbell, 2011; Clay, 2011; Clay *et al.*, 2011; Clay and Zuberbühler, 2012; Dixson, 2012; Liebal *et al.*, 2014). The adaptive significance of copulation calls is debated. They are thought to either concentrate paternity by inciting mate guarding behaviours or confuse paternity and incite sperm competition (Hamilton III and Arrowood, 1978; Hrdy and Hausfater, 1984; O'Connell and Cowlishaw, 1994; Cowlishaw and O'Connell, 1996; Henzi, 1996; Nikitopoulos *et al.*, 2004; Maestripieri and Roney, 2005; Pradhan *et al.*, 2006; Townsend, Deschner and Zuberbühler, 2008; Clay, 2011; Clay *et al.*, 2011; Clay and Zuberbühler, 2012). Either way they allow females to influence male behaviour and perceptions in ways necessary to prevent infanticide. Similarly, according to the graded signal hypothesis, the exaggerated sexual swelling, which is present during the oestrous cycle, signals the approximate timing of ovulation (Nunn, 1999). The extent of the swelling of the anogenital region, varies in relation to hormonal levels related to the point of the oestrous cycle. As

ovulation approaches maximum tumescence of the swelling is seen. Maximum tumescence occurs over multiple days and ovulation only lasts one day. Therefore, the exact timing of ovulation could occur at any point during or near maximum tumescence (Nunn, 1999; Zinner *et al.*, 2004). Added to this, the reliability and length of maximum tumescence varies between species. For example, maximum tumescence lasts longer in bonobos (*Pan paniscus*) and less reliably predicts ovulation than in chimpanzees (*Pan troglodytes*) (De Waal, 1997; Zinner *et al.*, 2004). Therefore, copulation calls, increased follicular phases and exaggerated sexual swellings in catarrhine primates allow females to maintain their counterstrategy of simultaneously biasing and confusing paternity (Hamilton III and Arrowood, 1978; Hrdy and Hausfater, 1984; O'Connell and Cowlishaw, 1994; Cowlishaw and O'Connell, 1996; Henzi, 1996; Nunn, 1999; Van Schaik, Hodges and Nunn, 2000; Nikitopoulos *et al.*, 2004; Zinner *et al.*, 2004; Maestripieri and Roney, 2005; Pradhan *et al.*, 2006; Townsend, Deschner and Zuberbühler, 2008; Clay, 2011; Clay *et al.*, 2011; Clay and Zuberbühler, 2012).

Genetics: Paternity Analysis And Microsatellite Markers

Although understanding the underlying strategies that mould sexual and reproductive behaviour, is important, equally important is a true understanding of the results of these reproductive strategies. Therefore, studies on behavioural ecology can elucidate many aspects of animal behaviour. Female mating success can be easily observed. Male reproductive success is much harder to determine, as it is impossible to observe conception. Due to this, observations on aspects of mating behaviour are used to approximate male mating success, but they cannot always reliably predict paternity (De Ruiter, Hooff and Scheffrahn, 1994). This is especially true in species where the females mate polyandrously. Behavioural observations can be made more reliable by studies on female hormonal status at times of mating. Throughout the oestrous cycle the likelihood of a female conceiving fluctuates, with peak fertility occurring around ovulation. Therefore, behavioural observations of

copulations combined with the hormonal timings of these copulations, can increase the confidence of the estimation an infant's paternity. For example, female black horned capuchins mated with alpha males during their periovulatory period of their cycle, making these males the most likely sire of resulting infants born in the following birth seasons (Tiddi, Wheeler and Heistermann, 2015a, 2015a). However, as females still mated with non-alpha males, albeit during less fertile phases of their cycle, one can only hypothesize about the paternity of these infants (Tiddi, Wheeler and Heistermann, 2015a, 2015b). For these reasons the field of genetics has become increasingly important in behavioural ecology and paternity analysis, as genetic paternity analyses can more successfully determine paternity. For example, in the monogamously pair bonded alpine marmot (*Marmota marmota),* female marmots predominantly mate monogamously with their pair bonded mate. However, 15-20% of the infants and about 30% of the litters were sired by EPMs (Goossens *et al.*, 1998; Cohas *et al.*, 2006).

Since the 1970s, genetic paternity analysis of wild populations has become increasingly widespread (Pope, 1990; De Ruiter, Hooff and Scheffrahn, 1994). As DNA extraction methods became more sophisticated, it became possible to use a wider variety biological tissues, like blood, faeces and hair (Pope, 1990; De Ruiter, Hooff and Scheffrahn, 1994; Pusey and Packer, 1994; Goossens *et al.*, 1998; Gerloff *et al.*, 1999; Constable *et al.*, 2001; Bellemain *et al.*, 2006; Feh and Munkhtuya, 2008; Muniz and Vigilant, 2008; Améndola-Pimenta *et al.*, 2009; Di Fiore *et al.*, 2009; Muniz *et al.*, 2010; Walling *et al.*, 2010; Dubuc *et al.*, 2011; Kim and Sappington, 2013; Van Belle, Estrada and Di Fiore, 2014; Van Belle *et al.*, 2014). Because hair and especially faeces can be obtained from a wild animal without causing distress (Constable *et al.*, 2001), these non-invasive techniques have become more widespread (Goossens *et al.*, 1998; Escobar-Páramo, 1999, 2000; Gerloff *et al.*, 1999; Constable *et al.*, 2001; Bellemain, Swenson and Taberlet, 2006; Muniz and Vigilant, 2008; Di Fiore *et al.*, 2009; Izar *et al.*, 2009; Muniz *et al.*, 2010; Van Belle, Estrada and Di Fiore, 2014; Van Belle *et al.*, 2014).

In population genetics and paternity analysis, the use of microsatellite markers is very common. Microsatellites are codominant, highly polymorphic, and with specific tandem repeats throughout

the genome (Escobar-Páramo, 1999, 2000; Morin *et al.*, 2001; Glenn and Schable, 2005; Kalinowski, Taper and Marshall, 2007; Muniz and Vigilant, 2008; Améndola-Pimenta *et al.*, 2009; Muniz *et al.*, 2010; Walling *et al.*, 2010; Dubuc *et al.*, 2011; Thomas and Bell, 2012; Kim and Sappington, 2013; Madesis, Ganopoulos and Tsaftaris, 2013). The codominant nature of microsatellites is very useful, as allows for heterozygosity to be studied. The polymorphic nature of microsatellites means that there are many alleles per locus which easily allows for individuals to be differentiated even when closely related (Escobar-Páramo, 1999, 2000; Morin *et al.*, 2001; Glenn and Schable, 2005; Kalinowski, Taper and Marshall, 2007; Muniz and Vigilant, 2008; Améndola-Pimenta *et al.*, 2009; Muniz *et al.*, 2010; Walling *et al.*, 2010; Dubuc *et al.*, 2011; Thomas and Bell, 2012; Kim and Sappington, 2013; Madesis, Ganopoulos and Tsaftaris, 2013). The specific tandem repeats are locus specific units of 1 to 6 base pairs (bp) which are present in loci across the genome. Furthermore, microsatellites can be amplified using very little DNA which makes them especially useful in non-invasive field studies (Goossens *et al.*, 1998; Escobar-Páramo, 1999; Glenn and Schable, 2005; Thomas and Bell, 2012; Kim and Sappington, 2013; Madesis, Ganopoulos and Tsaftaris, 2013; Tokuda, Martins and Izar, 2014).

The first step of microsatellite analysis involves building a microsatellite library which involves the identification of microsatellite, creation of primers (a small and single-stranded DNA section) and testing the utility of the microsatellite (Glenn and Schable, 2005; Muniz and Vigilant, 2008; Lin and Chang, 2013; Madesis, Ganopoulos and Tsaftaris, 2013). In microsatellite analysis, primers are specific DNA sequences which flank microsatellite repeats (Glenn and Schable, 2005; Lin and Chang, 2013; Madesis, Ganopoulos and Tsaftaris, 2013). These primers are needed for the amplification of microsatellites in PCR reactions (Glenn and Schable, 2005; Lin and Chang, 2013; Madesis, Ganopoulos and Tsaftaris, 2013; National Human Genome Research Institute (NHGRI), 2023).

The creation of microsatellite libraries and primers is very time-consuming, expensive and involves specialised knowledge. One way that researchers circumvent this problem is by testing out microsatellites across species. This can work as primer bonding sites can be similar in closely related

species, especially congeneric ones (Glenn and Schable, 2005; Muniz and Vigilant, 2008; Lin and Chang, 2013; Madesis, Ganopoulos and Tsaftaris, 2013; Tokuda, Martins and Izar, 2014). How closely related a species has to be for a marker to work depends on the microsatellite locus. For example, microsatellites developed for the same species (*Sapajus nigritus*), the same sub-family (Cebidae: *Cebus capucinus*) and same parvorder (Platyrrhini*: Leontopithecus chrysopygus*) were tested in black horned capuchins (Escobar-Páramo, 1999, 2000; Perez-Sweeney *et al.*, 2005; Muniz and Vigilant, 2008; Tokuda, 2012; Tokuda, Martins and Izar, 2014). Of the 17 microsatellites tested, 10 were successfully able to be used (Tokuda, 2012; Tokuda, Martins and Izar, 2014). Another problem with microsatellite analysis is that although genomically widespread, not all loci can be used. This is because they may not amplify during a PCR and/or may be monomorphic. In addition, alleles from microsatellite loci with similar amounts of base pairs can be hard to differentiate, and allele calling methods may differ across laboratories, making experiments harder to repeat and compare (Madesis, Ganopoulos and Tsaftaris, 2013).

Black Horned Capuchins (*Sapajus nigritus)*

Black horned capuchins have clear linear dominance hierarchies, which determine the results of food competitions, grooming relationships and reproductive success (Janson, 1984, 1985, 1996; Di Bitetti, 1997; Di Bitetti and Janson, 2001b; Tiddi, Aureli and Schino, 2010, 2012; Tiddi, Aureli, Polizzi di Sorrentino, *et al.*, 2011; Tiddi, Aureli, Schino, *et al.*, 2011; Janson, Baldovino and Bitetti, 2012; Wheeler, Tiddi and Heistermann, 2014; Tiddi, Wheeler and Heistermann, 2015a, 2015b). Male alpha tenures are long, lasting on average 5 years. Subordinate males have three methods of becoming alphas: (1) reproductive queuing (waiting for the current alpha to die/disappear) (2) challenging the current alpha and winning (3) becoming the alpha of a new female group formed after new male takeover (Muniz *et al.*, 2010; Janson, Baldovino and Bitetti, 2012). Furthermore, in the months following a new alpha takeover group composition changes, with most of the males emigrating from

the group. Daughter groups are also often formed, with females with vulnerable infants and some of their matrilineal relatives leaving the main group. These daughter groups formation are the result of a female counterstrategy and also act to prevent groups from becoming too big (Janson, Baldovino and Bitetti, 2012).

The black horned capuchin monkey offers a good opportunity to study female mate choice. This is because females perform proceptive displays to solicit copulations with males during their fertile periods. Furthermore, males have not been observed to show any mate guarding behaviour (B. Tiddi and B. Wheeler, personal communication). These displays conform to the predictions of the graded signal hypothesis, by advertising the day to day likelihood of ovulation (Nunn, 1999; Tiddi, Wheeler and Heistermann, 2015a, 2015b). These displays consisted of visual signals like chest rubbing and vocal calls. The visual displays more reliably indicated ovulation than the vocal calls. In the capuchins' arboreal environment, visual signals only broadcast for short distances. Therefore, visual proceptive displays would only be observed by individuals in close proximity to the females. This means that the target male, who is approached by the female and is only of the few receivers of the female's visual signal. This means that it is likely for other males and group members to miss these visual signals. Through this way, females can bias paternity towards preferred males. On the other hand, vocal signals travel further in an arboreal environment and so whole group is privy to the fact that the female is fertile. However, vocal signals less reliably indicated ovulation than visual signals. If a male mates with a female within a few days of hearing her calls, while she is making vocal proceptive displays and she conceives that cycle, from the male's perspective he is a likely sire (paternity confusion) (Tiddi, Wheeler and Heistermann, 2015a, 2015b; Bernaldo de Quirós *et al.*, 2018). Therefore, through proceptive displays females are able to bias paternity towards their preferred male while still confusing paternity to avoid infanticide.

Female black horned capuchins often prefer alpha males as mating partners (Janson, 1984; Ramírez-Llorens *et al.*, 2008; Izar *et al.*, 2009; Muniz *et al.*, 2010; Janson, Baldovino and Bitetti, 2012; Tiddi, Wheeler and Heistermann, 2015a, 2015b; Tiddi *et al.*, 2018). Females solicit alpha males during their

periods of peak fertility and only mate with subordinate males during their least fertile days of their oestrous cycle (Janson, 1984; Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012; Tiddi, Wheeler and Heistermann, 2015a, 2015b). Female alpha male preference was thought to be related the male's ability to monopolise food resources (Janson, 1984; Di Bitetti and Janson, 2001a; Izar *et al.*, 2009; Janson, Baldovino and Bitetti, 2012; Tiddi, Wheeler and Heistermann, 2015a, 2015b). However, it was found that manipulating the amount of alpha male control over food resources, did not affect female alpha male preference (Tiddi *et al.*, 2018). Therefore, this alpha preference must be related to over other factors like perhaps the long alpha tenures in the group, which can reduce some of the need for promiscuity.

Research Aims And Hypotheses

This study focused on the black horned capuchin (*Sapajus nigritus*). In regards to this species, much is known about female reproductive success but male reproductive success is only vaguely understood. Based on previous behavioural and hormonal studies, it is known that females mate preferentially during peak fertility with alpha males (Janson, 1984; Izar *et al.*, 2009; Tiddi, Wheeler and Heistermann, 2015a, 2015b; Tiddi *et al.*, 2018). Therefore, it can be safely assumed that alpha males are the most successful in siring infants. However, females still mate promiscuously with multiple other males during the less fertile days of their peri-ovulatory periods. This means that a proportion of non-alpha males would also sire infants. The only way conclusively determine individual male reproductive success is to perform genetic paternity analysis. The first hypothesis of this study, was whether male rank predicts paternity success (Objective 1). The Priority of Access Model posits that (1) higher ranking male have higher reproductive success and that (2) female synchrony affects the ability of males to monopolise females. To determine whether the distribution of genetic paternity was could be predicted by the PoA model, male rank and female synchrony values were combined into an expected success value (Objective 2). This expected success was then tested against observed success (based on paternity assignments). The null hypothesis (H_0) was tested using the Paired sample T-Test (with O: Observed, E: Expected).

$$H_0 = M_E - M_O = 0 \cdots \textit{Equation 1.}$$

$$H_1 = M_E - M_O \neq 0 \cdots \textit{Equation 2.}$$

Infanticide has been recorded in black horned capuchin monkeys (Ramírez-Llorens *et al.*, 2008). Therefore, female counterstrategies must have evolved, including paternity confusion. However, in this species, it is currently not known to what extent females are confusing paternity among non-alpha males, and whether they are confusing paternity enough to prevent infanticide. SSI was tested looking at male rank (non-alpha) and male ID (non-alpha) in this study using an adapted Van Schaik

(2000) infanticide prediction equation (Objective 3), where (tn = time to sire next infant without infanticide, ti = time to sire next infant after infanticide, P = probability to sire after infanticide and p = probability victim is killer's offspring):

$$[(tn - ti) \ / \ tn] \ P - p \cdots \textit{Equation 3.}$$

The hypotheses were as follows:

$$H_0 = [(t_n - t_i)/t_n]P - p = 0 \cdots \textit{Equation 4.}$$

$$H_1 = [(tn - t_i) \ / \ t_n]P - p \neq 0 \cdots \textit{Equation 5.}$$

By using microsatellite markers developed for same family (Cebidae from *Cebus capucinus*) in *Sapajus nigritus*, further insight will be gained in efficacy of using markers across species (Objective 4). In addition, the faecal and hair samples were over 10 years old at the time of extraction. This study briefly and qualitatively explored how viable using older faecal samples is, and whether they produce reliable results (Objective 5).

Methodology

Fieldwork

Study Site And Subjects

Capuchin classification has varied over the years. In the first classifications, capuchin monkeys were recognised as one genus *Cebus,* with had two types, tufted (later robust) and untufted (later gracile). Tufted capuchins were classed as *Cebus apella*, with 6-7 subspecies, while untufted capuchins were assigned to three species: *Cebus capucinus, Cebus albifrons*, and *Cebus nigrivittatus (*later *olivaceus*) (Lynch Alfaro *et al.*, 2012; Lynch Alfaro, Silva Jr and Rylands, 2012; Ruiz-García *et al.*, 2012; Ruiz-García, Castillo and Luengas-Villamil, 2016). In 2001, *Cebus apella* as the only robust capuchin species was reviewed, with 4-7 subspecies being granted species status including *Cebus nigritus.* It was even argued that robust capuchins should be belong to their own genus, *Sapajus*. However, it was not until 2012 that this was more widely used (Lynch Alfaro *et al.*, 2012; Lynch Alfaro, Silva Jr and Rylands, 2012; Ruiz-García *et al.*, 2012; Ruiz-García, Castillo and Luengas-Villamil, 2016).

The Phylogenetic Species Concept (PSC) has been used to divide *Sapajus apella sensu stricto* into 6 geographical sub-clades which combine morphologically classified species together, for example the Peruvian/Brazilian sub-clade of S. *apella/macrocephalus* (Lima *et al.*, 2017). Janson (1984, 1985) studied the brown capuchin (then *Cebus apella*) in Manu National Park, Peru. Its current designation is confusing as it has been listed as *Cebus apella peruanus* (Lee and Huang, 2021; *Manu, Manu National Park | Parque Nacional del Manu*, no date), *Sapajus macrocephalus/ Cebus apella macrocephalus* (Lynch Alfaro *et al.*, 2012; Lynch Alfaro, Silva Jr and Rylands, 2012; Ruiz-García *et al.*, 2012; Ruiz-García, Castillo and Luengas-Villamil, 2016) or S. *apella/macrocephalus* (Lima *et al.*, 2017).

The black horned capuchin (*Sapajus nigritus* previously known as *Cebus apella nigritus* and *Cebus nigritus)* is an Atlantic Forest based primate found in north-eastern Argentina and south-eastern

Brazil (Izar *et al.*, 2009; Lynch Alfaro *et al.*, 2012; Lynch Alfaro, Silva Jr and Rylands, 2012; Ruiz-García *et al.*, 2012; Tokuda, 2012; Ruiz-García, Castillo and Luengas-Villamil, 2016; Lima *et al.*, 2017). The study site is the humid and sub-tropical region of Iguazú National Park in Argentina (25°40' S, 54° 30' W). Black horned capuchin monkeys are omnivores, who are mainly frugivore-insectivores, especially during the austral summer (October to January). In the austral winter (July to August) seasonal variation in temperature and day length affects the abundance of fruits and insects (Brown and Zunino, 1990; Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012; Tiddi, Wheeler and Heistermann, 2015a; Tiddi *et al.*, 2018). During this period of scarcity, fallback foods become the main staple of these monkeys' diets (Brown and Zunino, 1990; Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012). This population has been studied since 1988 and provisioned for since 1992 (Janson, 1996; Janson, Baldovino and Bitetti, 2012). Between 1992 and 1994 provisioning occurred between the months of May and August (Di Bitetti and Janson, 2001a). However, this appears to have affected birth rates as two females with surviving offspring were seen to reproduce in three successive years versus the norm of approximately every two year. From 1995 until present, provisioning occurs between June and August when fruits are most scare (Di Bitetti and Janson, 2001a). In Iguazú, 1m by 1m wooden platforms with cut up fruit, originally tangerines and currently bananas, were suspended up to 10m. The numbers of fruit pieces and platforms provided depends on the aims of the study (Janson, 1996; Wheeler, Tiddi and Heistermann, 2014; Tiddi *et al.*, 2018).

Sapajus nigritus lives in multifemale-multimale groups polygynandrous mating, which range from 7 to 44 individuals (Janson, Baldovino and Bitetti, 2012). Female philopatry exists resulting in matrilines with close kinship bonds and agonistic support. Adult daughters inherit close ranks to their mothers, meaning matrilines can be assorted into dominance hierarchies (Janson, Baldovino and Bitetti, 2012). Alpha matrilines tend to remain the highest ranking until the death of the matriarch. Females of alpha matrilines have significantly earlier ages at first birth than lower ranking matrilines. However, being an alpha matriline did not relate to higher overall fecundity (Janson,

Baldovino and Bitetti, 2012). To avoid inbreeding, males disperse during early adulthood, usually between the ages of 5 and 7 (Janson, Baldovino and Bitetti, 2012).

Additionally, black horned capuchins are a seasonally reproductive species, although females can technically be sexually receptive throughout the year (Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012). The majority of mating occurs between May and September (during periods of food scarcity). Peri-ovulatory cycles last for approximately 21 days (Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012). Proceptive displays which characterise the peri-ovulatory phase last for approximately 5 days. Often females undergo multiple cycles before conception (Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012). Gestation lasts for approximately 155 days. Therefore, the birth season occurs between October and February (during periods of food abundance) (Di Bitetti and Janson, 2001a; Janson, Baldovino and Bitetti, 2012).

Grooming varies seasonally with the lowest rates occurring during winter and the period of food scarcity, when less time is spent socialising and resting (Di Bitetti, 1997). The amount of grooming an individual participates in depends on rank, age class and sex. Dominant individuals receive the most grooming, with the alpha male and alpha female being the most common grooming dyad (Di Bitetti, 1997). Subordinates often groom dominants to obtain increased tolerance during feeding (Tiddi, Aureli, Polizzi di Sorrentino, *et al.*, 2011; Tiddi, Aureli and Schino, 2012). Females were more likely to groom higher ranking females in line with the predictions of the Seyfarth model (Seyfarth, 1980; Tiddi, Aureli and Schino, 2012). This results in females mainly grooming other females of adjacent ranks and lower ranking females being involved in the much less grooming bouts Adult subordinate and juvenile males and infants received the least grooming (Tiddi, Aureli and Schino, 2012). Females are the most common receivers of grooming, and are more likely to reciprocally groom each other, potentially because females are philopatric and more closely affiliated with each other than they are with males (Di Bitetti, 1997). In addition, after females give birth to an infant, they receive significantly more grooming; other females groom the mothers in order to hold the infant (Di Bitetti, 1997; Tiddi, Aureli and Schino, 2010). Fertile females spend more time grooming adult males,

especially focusing on alpha males (Di Bitetti, 1997). However, during the non-breeding season, females do not prefer alpha males as grooming partners. Females, especially the alpha female, instead maintain close proximity with the alpha male (Tiddi, Aureli, Schino, *et al.*, 2011).

Behavioural Sampling

Fieldwork for this study was conducted by a team lead by Drs Barbara Tiddi and Brandon Wheeler between May 2010 and August 2014, during the mating seasons of these years (Tiddi, Wheeler and Heistermann, 2015a). Throughout the rest of the year, especially the birth seasons, field assistants and other researchers would observe the groups every few weeks. Three fully habituated groups, Macuco, Guenon (also known as Rita and commonly referred to Rita in the literature, but referred to as Guenon in this paper) and Spot, with easily distinguishable members (based on fur colouration, facial features etc.) were followed. Furthermore, due to the longitudinal nature of the study information such as group demography and infant births came from research in previous years, especially between 2004 and 2010. This research was conducted by a variety of research teams (Di Bitetti and Janson, 2001a; Ramírez-Llorens *et al.*, 2008; Wheeler, 2009, 2010; Tiddi, Aureli and Schino, 2010; Janson, Baldovino and Bitetti, 2012; Scarry and Tujague, 2012a; Scarry, 2013).

Focal and ad libitum behavioural sampling (Tiddi, Wheeler and Heistermann, 2015a; Bateson and Martin, 2021) was conducted during the mating seasons, between the hours of 07:00 and 18:00, by Drs Barbara Tiddi and Brandon Wheeler with the help of field assistants. Most times two (always at least one) researchers/assistants were watching a group, with behaviours of interest being noted. These included: (1) Male interactions with: (a) adult male group members leading to male dominance hierarchy determinations (based on data collected during the mating season) and (b) adult females especially when females were fertile and cycling. Dominance was calculated based on competition over resources, often food resources; as the feeding platforms used, allowed for the

observance of such relationships (Janson, 1985, 1996; Wheeler, Tiddi and Heistermann, 2014). Any agonistic encounters, successful displacements and any other dyadic interactions which lead to winners and losers, contributed to dominance hierarchy determination (Janson, 1996). In addition, female proceptive behaviour and corresponding sexual behaviour, was recorded with details on the individuals involved. While females were making proceptive displays, attempts were made for them to be followed throughout those days. Proceptive females were also the focus of focal sampling bouts of 30 minutes, with attempts to do focal samples on the same individual in the morning and afternoon (Tiddi, Wheeler and Heistermann, 2015a). During the non-mating seasons, during regular (every 1-3 weeks), groups were checked on by field assistants. In particular, group membership and individuals present were noted. Any changes to group composition like male appearances, male dispersals and/or infant births were noted for that visit and the timeline of these were estimated based on the group composition at the last sighting. For example if field assistants noticed a new infant a week after their last visit, the infant's birth was estimated to be within that week.

Faecal And Hair Collection

In total 77 individuals were followed and genetic samples were collected for 70 individuals across all three groups. Samples for 23 monkeys across the study years were obtained from Guenon. These 23 monkeys consisted of: six adult females (1 female was not sampled), six adult males (1 male was not sampled) and 11 Infants (including Eduardo and Sergio who immigrated to Macuco as adults). In Macuco there were samples from 35 monkeys including: 8 adult females (1 female was not sampled), 9 adult males (4 males were not sampled) and 18 infants. In addition, five monkeys broke Macuco to form the daughter group Spot including: 3 adult females and 2 adult males. In Spot, samples from 16 monkeys: 4 adult females, 3 adult males and 9 infants were obtained. Group membership varied between the years. This study was approved and complied to the ethical standards of the German Primate Center and the National Parks Administration of Argentina and

Argentinian laws (Wheeler, Tiddi and Heistermann, 2014; Tiddi, Wheeler and Heistermann, 2015a; Tiddi *et al.*, 2018).

Hair samples were collected using short-blunted wooden dowels designed for non-invasive sampling (Améndola-Pimenta *et al.*, 2009). Dowels with glue on the tip of them, were blown through a cooper blowpipe, from a distance of 5-10m at a monkey. The dowel would hit, stick to the monkey's fur and would be pulled off and dropped to the ground by the monkey (Améndola-Pimenta *et al.*, 2009). Most monkeys reacted to the dowel as if they had been stung (Améndola-Pimenta *et al.*, 2009; B. Wheeler, personal communication). Using latex gloves, researchers/assistants would then place the hair sample into a labelled polypropylene tube. It was attempted to collect hair samples from all individuals across all three groups. Individuals were normally darted once, as long as five or more hairs were collected. If less hairs were collected then an individual was darted a maximum of two more times. Monkeys were not darted more than 3 times over a four month period (B. Wheeler, personal communication). In total 65 hair samples were obtained from individuals across the years from 3 study groups. Faecal samples not contaminated by urine and from clearly identified group members were collected within 30 minutes of defaecation. These samples were collected opportunistically. Samples were retrieved using latex gloves and placed in tubes containing RNAlater (which preserves DNA). 275 faecal samples from the three groups, numbering from 1 to 5 samples per individual, were collected between 2012 and 2014 (Van Belle, Estrada and Di Fiore, 2014; Wheeler, Tiddi and Heistermann, 2014; Tiddi, Wheeler and Heistermann, 2015a).

Both hair and faecal samples were brought to the field station at the end of the study day, where they were stored at room temperature. By the end of 2014, these samples were shipped to the Genetic Laboratory at the German Primate Center, Göttingen where they were frozen and stored at -18°C. 12 DNA extractions from different study subjects were shipped to Dr Anthony Fiore's lab at the University of Texas (Austin, the United States of America). Dr Fiore's lab was able to successfully amplify these extractions across 9 markers, demonstrating that the majority of samples had amplifiable DNA at multiple loci. In 2018, the hair and faecal samples were shipped on dry ice from

Germany to the DICE lab at Kent. The samples were then stored in the freezer at -18°C. Labwork on this project was performed by Dr Tiddi in 2019, by MMS and Dr Tiddi during a handover period in the summer of 2021. From September 2021, MMS (Maya Marriott-Semper: Principal Investigator) was primarily responsible for completing the project, with Dr Tiddi acting as one of the supervisors.

Lab work

Microsatellite Library

The microsatellite screening process consisted of running: (1) extractions, (2) Polymerase Chain Reactions (PCRs), (3) checking gels for peaks, (4) sending the plate for genotyping (to Durham), and (5) calling peaks in Geneious (see following sections for detailed information about each step). Dr Tiddi started the screening with 18 microsatellites which were successfully amplified in *Cebus capucuinus* (Muniz and Vigilant, 2008). All screening steps were completed on these 18 microsatellites. In addition, these markers were tested further validated by blood samples from 4 Port Lympne individuals from the sister genus species of black capped capuchin (*Sapajus apella*). From this screening process, 16 of the 18 microsatellites were chosen to be used in this study as they amplified successfully and consistently enough. These 16 markers are: Ceb02, Ceb03, Ceb04, Ceb07, Ceb08, Ceb09, Ceb10, Ceb105, Ceb11, Ceb115, Ceb119, Ceb121, Ceb127, Ceb128, Ceb130 and D7S794 (originally from humans) (

, Table 3 & Table 4). Ceb01 and Ceb120 were the two unused markers. 1 microsatellite, Ceb128 was removed from analysis during post lab-work genotyping as it was determined to be monomorphic.

Additionally, in 2019 Dr Tiddi began screening markers from newer studies and/or more closely related species (Escobar-Páramo, 1999, 2000; Tokuda, 2012; Tokuda, Martins and Izar, 2014; Van Belle, Estrada and Di Fiore, 2014). 5 markers which were successfully amplified in: (1) the Iguazú population (same as this study's population) of *Sapajus nigritus* (Escobar-Páramo, 1999, 2000), (2) the Macarena, Columbia population of *Sapajus apella* were screened (Escobar-Páramo, 1999, 2000). 7 markers were screened (the same 5 microsatellites plus another 2) that were successfully amplified in *Sapajus nigritus* population Brasil (Tokuda, 2012; Tokuda, Martins and Izar, 2014). Also in 2019, Dr Tiddi started testing 7 markers which were successfully amplified in black howler monkeys (Alouatta pigra) (Van Belle, Estrada and Di Fiore, 2014). The screening of the 14 markers,

first tested in 2019, only progressed to step 4, as the peaks were never called in Geneious due to the time constraints of the project.

Overall Lab Techniques Used

On entering the lab, a lab coat and small or medium nitrile gloves were put on, to be worn throughout the lab session. Gloves were changed as often as necessary, including when changing between activities like from making a gel to making a plate. If there was any suspected contamination, for example any liquid spilled, especially from a DNA containing tube, gloves were immediately changed. Gloves contaminated with bleach were also immediately changed to reduce the possibility that DNA could be destroyed. At the beginning of the lab session, surfaces to be used were wiped down with 10% bleach and blue roll. Gloves were changed and blue roll was laid out on surface to be used. Pipettes were UV radiated for 10 minutes, 5 minutes each side. Any other lab equipment that may come in contact with the samples, like tubes, PCR plates, tube holders, scissors, scalpels was also UV radiated for 5 minutes. Pipettes were also cleaned, by being wiped down with 10% bleach and then 100% ethanol, every few weeks. As much as possible, the order of tips pipette tip boxes was matched to the order of samples on a Polymerase Chain Reaction (PCR) plate. When pipetting DNA containing liquids, 1 tip per sample was used. When the same non-DNA containing liquid was pipetted into an empty tube, the same tip was used for every 4 samples. If the tube had DNA containing liquid in it and the non-DNA containing liquid was being pipetted into the tube; either 1 tip was used per sample or the same tip was used to carefully pipette (not touching the tube) into multiple (3 to 4) samples. All tips were disposed into a plastic beaker that was then emptied into the bin at the end of the session. Tips that came in direct contact with biological matter i.e faeces, blood or hair, were left overnight under the fumigation hood soaking in a 10% bleach solution. The next day the tips were disposed in the bin. At the end of the session, all used work stations were wiped down with 10% bleach, all tips were thrown away, pipettes were returned to

their holders All washable and reusable lab equipment was washed with water and put on drying rank. Double distilled water was made by autoclaving distilled water.

Every laboratory experiment was dated and recorded into a laboratory notebook which was used by both Dr Tiddi and MMS for the whole experiment. All extractions, PCRs, Gels and Plates were numbered based on order of occurrence, i.e extraction 1 came before extraction 2 etc,. At all stages, samples/individuals and markers used, changes to protocol, potential contamination and any other important notes were recorded. For extractions, names and codes of individuals, numbers assigned to each individual, sample composition, potential contaminations and any protocol changes were written out. For PCRs the design of the plate including individual IDs (and a negative control denoted NC), amounts of master mix components used: including marker primers, Taq and double distilled water and any errors or changes to plate design were noted. For Gels, the order of samples and ladder position, marker tested and any potential contamination or method changes were all recorded. For plates, records for each well included: sample ID code, marker, dilution and extraction number. PCR numbers were also included in the title to help keep track of samples genotyped. During plating any design changes, including small quantities of PCR product or potential contaminations were recorded (Appendix B. PCR and Gel Tracker).

4 sizes of Gilson single channel pipettes were used. These included: (1) red tops with a range of 0.1 to 10 µl, (2) cream tops ranging from 2 to 20 µl, (3) yellow tops ranging from 20 to 200 µl and (4) blue tops ranging from 50 to 1000 µl. 2 sizes of BioPette's multichannel pipette were used: (1) the blue one at 0.5 to 10 µl and (2) the purple one from 20 to 200 µl. Multichannel pipettes were only used in the making of plates to be sent to Durham University. A mixture of filtered and non-filtered tips were used depending on availability in the laboratory.

Extractions

71 individuals (Appendix A. Study Subjects) were genotyped across 16 tetranucleotide microsatellites. 1 infant (Mora) was removed during paternity analysis, as the same individual had been sampled twice under different names (Omar and Mora). During the screening process Dr Tiddi helped by MMS, genotyped 35 individuals across 16 markers. Of these 35 individuals, 4 (1 male: Moose (M) and 3 females Nancy (N), P1842865 (P18) and Wilma (W) were from blood FTA cards of *Sapajus apella*. This leaves the 31 black horned capuchin monkeys whose faecal and hair samples were genotyped. Of them, 7 individuals had 2-5 repeats with different faecal (n=17) and hair (n=3) samples being tested. The remaining 24 individuals only had 1 repeat and were extracted from faecal samples. After analysing the genotyped data and comparing the repeats which produced variable results with many mismatches; the following lab work goal was decided on: for one individual, one faecal sample was to be extracted 3 times with 3 PCRs being run from these extractions. Using one sample per individual was decided to eliminate errors and mismatches between samples of the same individual which might be due to potential that faecal samples had been incorrectly assigned during sample collection. Additionally, hair samples were no longer used from this point on as they produced much more variable and sporadic results than the faecal samples. These changes were implemented from January, 2022. In this project, across 16 markers, faecal samples from 70 monkeys were extracted and thus repeated at least twice with the range of repeats for an individual being from 2 to 6.

In total 15 extractions, plus 1 blood extraction of FTA cards, were performed over the duration of this project. Extractions 1 (with 10 faecal samples plus 2 hair samples which were extracted separately), 2 (with 7 samples) and 3 (with 11 samples) were performed on faecal samples by Dr Tiddi in 2019. For extraction 1, two sets of PCRs, gels, plates and Geneious analyses, were run across 16 microsatellites. Some samples from extractions 2 and 3, also had a PCR-level repeat for only 8 microsatellites (Ceb02, Ceb04, Ceb07, Ceb09, Ceb10, Ceb11, Ceb121 and Ceb130). All 16

microsatellites could not be repeated, as extraction samples ran out before this. In the summer of 2021, extraction 4 was completed on 5 hair samples (this extraction was never tested or genotyped) by Dr Tiddi and MMS. Faecal extractions 5-15 were carried out by MMS (5 was done with the help of Dr Tiddi) between January and June of 2022. A previously unopened Qiagen QIAamp DNA Stool Mini Kit from 2018, was used in extractions 5 and 6. Extraction 5 was done in two batches (batch 1: 10 samples, batch 2: 5 samples for a total of 15 samples). In extraction 6, five samples were extracted. The faecal samples in both extractions 5 and 6 were the same 20 (of the 24 samples needing a second repeat) samples used for extractions 1 to 3. Therefore, extractions 5 and 6 were the original attempt of a second repeat from extraction level. However, these extractions did not work well as gel images from PCRs performed on these extractions showed very few bands and thus that the DNA did not amplify well in the PCRs. For extraction 5, 8 PCRs (using old primer working stocks) on 5 microsatellites, Ceb130: done twice, Ceb120 and Ceb128: done twice with second time 4 µl DNA added and Ceb11 and Ceb121, were run. In 3 PCRs (Ceb121, Ceb130 and Ceb128-4µl) no bands were present on the gel. For the remaining 5 PCRs (Ceb130, Ceb120, Ceb128, Ceb11 and Ceb120-4µl) 1 to 4 (of 15 potential) bands were present. For extraction 6, twelve PCRs, with newly made primer working stocks, were performed on 12 markers. Of these 12, 7 PCRs (Ceb02, Ceb11, Ceb121, Ceb130, Ceb115, Ceb09 and Ceb127) had no bands present at all. The remaining 5 PCRs (Ceb07, Ceb08, Ceb03, Ceb105 and Ceb128) had 1-2 (of 5) bands present. To troubleshoot this problem, MMS tried using double the DNA (4µl vs 2µl), making new working stocks and 2 PCR tests with Ceb02 and Ceb07 on extractions 1 to 4 which worked properly with bands appearing in more than half of the samples (n= 14). This determined that: (1) the extractions contain a very low quantity of DNA and (2) the primers and whole PCR process is working properly. Due to this, it was decided that the kit was not working and to try a new kit.

Therefore, from this point on all faecal extractions (7a-15) were done using the Qiagen QIAamp Fast DNA Stool Mini Kit. Extractions 7a (n= 7), 7b (n= 13) and 7c (n= 4) were the second repeat for the 24 previously unrepeated individuals and the same faecal samples were used from extractions 1 to 3.

For extractions 8 to 15, 40 individuals and samples were extracted twice for two extraction level repeats. Extraction 8 was repeated by extraction 12 (both n= 8). Extraction 9 was repeated by extraction 13 (both n=11). Extraction 10 was repeated by extraction 14 (both n=11). Extraction 11 was repeated by extraction 15 (both n=10). Extractions 14 and 15 did not amplify well with many Geneious wells having no peaks. Therefore these 20 individuals should have been repeated again (not possible due to time and budget constraints of the project).

Throughout the study, a variety of extraction methodologies and different Qiagen kits were used. For extractions, in the first and last steps, tubes were labelled with 3 letter name code for the individual the sample was taken from and a number which was assigned to individual based on their assigned number for the extraction. For the rest of the steps, samples were just labelled with their extraction assigned number. Step 1 in all the extractions, when blood, hair and faecal samples were opened/exposed, was conducted under the fumigation/extraction hood. After extractions were completed, all samples were placed in a labelled box the freezer (-15°C) until needed.

After a failed (low DNA volume) extraction of the FTA cards, the cards underwent a purification and TE-1 wash. This was done via the following steps:

1. 2-4 punches of 2-4 mm each were taken from each card
2. The punches were placed into 1.5ml Eppendorf tubes
3. 200 µl of FTA Purification Reagent was added to each tube
4. The tube was flicked and shook
5. The tubes were incubated at room temperature for 5 minutes
6. All used FTA Purification Reagent was pipetted out and discarded
7. Steps 3-5 were repeated twice, for a total of 3 washes
8. 200 µl of TE1 buffer was added
9. The tubes were incubated at room temperature for 5 minutes
10. All used TE1 buffer was pipetted out and discarded
11. Steps 7-9 were repeated once, for a total of 2 washes

12. Tubes were dried on a heat block for 30 minutes

The purification and wash was then followed by an extraction using a Qiagen DNeasy Blood and Tissue Kit. The 8 step Qiagen protocol entitled purification of total DNA from animal tissues (spin-column protocol) was followed, as the FTA cards were treated as tissue. The steps were as follows:

1. Purified and washed FTA card pieces were placed into a 1.5 ml microcentrifuge tube with tweezers. 180 µl of Buffer ATL was added.
2. Then 20 µl of Proteinase K was added. The tube was closed and its contents were mixed thoroughly by vortexing. The sample was incubated at 37°C overnight.
3. The following day, thee sample was vortexed for 15 seconds. 200 µl of Buffer AL was added to the sample and then it was mixed via vortexing. 200 µl of 100% ethanol was added and the sample was again mixed by vortexing.
4. The mixture from step 3 was pipetted into a kit provided spin column which was in a 2 ml collection tube. For 1 minute the sample was centrifuged at ≥6000 x g (7000 rpm). The flow-through and collection tube was discarded.
5. The spin column was transferred to a new 2 ml collection tube and 500 µl of Buffer AW1 was added. For 1 minute the sample was centrifuged at ≥6000 x g (7000 rpm). The flow-through and collection tube was discarded.
6. The spin column was transferred to a new 2 ml collection tube and 500 µl of Buffer AW2 was added. For 3 minutes the sample was centrifuged at 17000 x g (13300 rpm). The flow-through and collection tube was discarded.
7. The spin column was transferred to a new labelled 2 ml collection tube and 200 µl of Buffer AE was added to the centre of the spin column membrane. The sample was incubated at room temperature for 5 minutes. For 1 minute the sample was centrifuged at ≥6000 x g (7000 rpm) to elute.
8. The elution process from step 7 was repeated to increased DNA yield.

The 8 step protocol from the Qiagen DNeasy Blood and Tissue Kit was also used for hair extractions, which included two individuals from extraction 1 (Maw and Bianca) and 5 individuals from extraction 4 (not genotyped). The following modifications to the procedure were implemented:

1. Step 1 modification: Hair samples were removed via tweezers from their tubes onto a petri dish. Hair was then cut up into smaller pieces to maximise lysis efficacy. Approximately 50mg (or a minimum of 10) of the least gluey hairs were added to a 1.5 microcentrifuge tube. 360 µl of Buffer ATL was added.
2. Step 2 modification: 40 µl of Proteinase K was added and the sample was incubated at 56°C overnight.
3. Step 7 modification: 75 µl of Buffer AE was added and the sample was incubated at room temperature for 30 minutes.

All faecal extraction centrifugations were performed at room temperature (15-25°C) at 17,000 x g (13,300 rpm). During centrifugations, all tubes were closed. If there was an uneven number of samples being extracted, a 2ml water filled microcentrifuge tube was centrifuged opposite to it, to keep the counterbalance system in place. Extractions 1 to 3 and 5 to 6 were performed using the Qiagen QIAamp DNA Stool Mini Kit. A Qiagen protocol that was adapted by Dr Fiore for platyrrhine faecal samples was followed. The protocol was as follows:

1. The end of the pipette tips were cut and 200 µl of faecal sample, with both faecal particles and RNAlater liquid, was pipetted into a 2 ml microcentrifuge tube. In extraction 5, for 3 samples (Estela, Octavio and Yoli) 3 different aliquots per sample were made and done in parallel until step 13 when all 3 aliquots of the same sample were added to the same spin column.
2. 1.6 ml of Buffer ASL was added to each faecal sample/aliquot. The samples were then vortexed continuously for 1 minute (or longer if not thoroughly homogenised). In an oven set to 56°C, the samples were lysed overnight on a rocking platform.

3. The next day, the heat block was warmed up to 70°C and Buffer AE was placed on top to heat to 70°C . The samples were taken out of the oven and centrifuged for a minute to pellet the stool particles.
4. 1.4 ml of the supernatant was pipetted into a new 2 ml microcentrifuge tube and the pellet was discarded.
5. 0.5-1 InhibitEX Tablet was added to each sample and vortexed immediately and continuously for 1 minute (or longer if the tablet was not completely suspended). The suspensions were incubated at room temperature for 1 minute which allowed the inhibitors to be absorbed by the InhibitEX matrix.
6. The sample was centrifuged for 6 minutes to pellet stool particles and inhibitors bound to the InhibitEX matrix.
7. Immediately following centrifugation, all the supernatant was pipetted into a new 2 ml microcentrifuge tube and the pellet was discarded. If too much time was taken or the pellet was accidentally pipetted, the sample was centrifuged for a further minute. The sample was then centrifuged at full speed for 3 minutes.
8. 30 µl of proteinase K was pipetted into a new 2 ml microcentrifuge tube.
9. 600 µl of the supernatant from Step 7 was pipetted into the tube containing proteinase K.
10. 600 µl of Buffer AL was added and vortexed for 15 seconds.
11. The samples were incubated in a heat block at 70°C for 30 minutes. During this time, every 10 minutes the samples were vortexed for 1 minute.
12. 600 µl 100% ethanol was added to the lysate and mixed by vortexing.
13. 600 µl of the lysate was added to a QIAmp spin column being careful not to moisten the rim. The spin column cap was closed and was centrifuged for 1 minute. The spin column was placed into a new 2 ml collection tube and the tube containing the filtrate was discarded.
14. The spin column was carefully opened, a second aliquot of 600 µl lysate was applied and then centrifuged for 1 minute. The spin column was placed in a new 2 ml collection tube and the tube containing the filtrate was discarded.

15. Step 14 was repeated with the third aliquot.
16. The spin column was carefully opened and 500 µl of Buffer AW1 was added. The cap was closed and the column was centrifuged for 1 minute. The column was placed in a new 2 ml tube and the filtrate was discarded.
17. After the cap was carefully opened, 500 µl of Buffer AW1 was added, the cap was closed and the spin column was centrifuged for 3 minutes. The tube containing the filtrated was discarded.
18. The spin column was placed into a new 2 ml collection tube, while the old collection tube was discarded, and was centrifuged at full speed for 1 minute.
19. The spin column was transferred into a new labelled 1.5 ml microcentrifuge tube and 75-100 µl of Buffer AE was pipetted directly onto the QIAamp membrane. The cap was closed and the samples were incubated at room temperature for 15-30 minutes.

Faecal extractions 7a to 15 used the original protocol from the Qiagen QIAamp Fast DNA Stool Mini Kit. The procedure was as follows:

Preparation: On opening the kits, ethanol was added to Buffers AW1 and AW2. The heat block was turned on so it had a chance to reach 70°C before it was needed. Buffers AL and InhibitEX were placed on the heat block as it was heating up to redissolve any precipitates. Faecal samples were taken out of the freezer and left to defrost for about 20 minutes (while pipettes and equipment were being cleaned and tubes were being labelled).

1. Pipette tips were cut to allow for the faecal samples to be more easily collected. 200 µl of faecal sample was pipetted into a 2 ml microcentrifuge tube.
2. 1 ml of InhibitEX Buffer was added to each stool sample. The sample was vortexed for 1 minute or more if the sample was not homogenized.
3. 25 µl of Proteinase K was pipetted into a new 2 ml Eppendorf tube.

4. The sample was centrifuged for 1 minute to pellet stool particles. If there were still particles in the supernatant, the sample was centrifuged again for a maximum of 3 centrifugations total at this step.
5. 600 µl of the supernatant was pipetted into the 2 ml tube containing Proteinase K.
6. 600 µl of Buffer AL was added and vortexed approximately 15 seconds to mix the sample.
7. The sample was incubated at 70ºC for up to 2 hours and 30 minutes (between 10 minutes and 2 hours and 30 minutes, normally for 2 hours). After incubation, the samples were briefly centrifuged in a Thermo Scientific mySPIN Mini Centrifuge, to remove drops from the inside lid of the tube.
8. 600 µl of 100% ethanol was added to lysate, and mixed by vortexing. The lysate was then briefly centrifuged in the mini centrifuge to remove drops from the inside lid.
9. 600 µl of lysate was carefully applied to the QIAamp spin column. The cap was closed and was centrifuged for 1 minute. The spin column was placed into a new 2 ml collection tube and the tube containing the filtrate was discarded.
10. Step 9 was repeated (normally 2 times) until all the lysate was loaded on the column. If following centrifugation, the lysate had not completely passed through the column, the sample was centrifuged again until the spin column was empty. The filtrate was discarded and the column was placed into a new 2 ml collection tube.
11. The spin column was opened and 500 µl of Buffer AW1 was added. The sample was centrifuged for 1 minute, the spin column was placed into a new 2 ml collection tube and the tube containing the filtrate was discarded.
12. The spin column was opened and 500 µl of Buffer AW2 was added. The sample was centrifuged for 3 minutes and the tube containing the filtrate was discarded.
13. The spin column was placed into a new 2 ml collection tube and the tube containing the filtrate was discarded. The sample was centrifuged for 3 minutes.
14. The spin column was transferred into a new labelled 1.5 ml microcentrifuge tube and 200 µl of Buffer ATE was pipetted directly onto the QIAamp membrane. The sample was incubated

at room temperature for a minute and then centrifuged for 1 minute to elute DNA. The spin column was discarded and the sample was placed in a labelled box in the freezer (-15°C).

Polymerase Chain Reactions (PCRs)

From 2019 (when method was established) 120 single plex PCRs were run. PCRs 1 to 43 to were completed by Dr Tiddi, PCRs to 42 to 49 were performed by Dr Tiddi and MMS together. PCRs 50 to 120 were done by MMS. Lab preparation steps were completed (see above). Before the 96-well PCR plate was UV radiated, it was cut down to the number of samples present (to the nearest column). For example, if there were 21 samples being tested, the plate would be cut down to 24 wells, with the extra 3 wells remaining empty. In addition, from PCR 50, when MMS took over lab work, all wells were labelled with sample ID code, to better keep track while pipetting. PCRs plates were UV radiated after being cut and before or after being labelled depending on when PCR design was written in the lab book. A mixture of primers bought in Germany (circa 2013/study start date) and primers purchased in 2019 from the UK were used. Working stocks of forward and/or reverse primers were made as necessary. A 1.5 ml Eppendorf was UV radiated, labelled with primer name, direction (forward (F) or reverse (R)), stock from (UK or German), Fluorescent Label (FL or not: in cases of F primers) and date created. From February, 2022 working stocks older than 3 months were not used. Working stocks were made at a 1:10 dilution, with 90 µl double distilled water to 10 µl of primer F or R.

Working stocks of primers were used in the PCRs. While waiting for lab equipment to be cleaned, working stocks of the both primers and DNA extractions to be used were removed from the freezer to defrost in their tubes. 1 µl of forward microsatellite primer, 1 µl of reverse microsatellite primer, 1 µl of double distilled water and 5 µl of MyTaq HS Red Mix (from Meridian Bioscience) was added to each sample in form of 8 µl from the single plex master mix. One master mix was made per microsatellite, and up to 6 master mixes were made for different markers during 1 session. To make the master mix, first the volumes of each component needed was calculated. This was done by

determining the number of samples plus the 1 negative control (N), and adding 4 to this value (for a few PCRs 2 was added in an attempt to minimize master mix wastage but this ended up in needing more master mix to be made). This value (n + 4) was then multiplied by the amount of each component present per sample. For example if there were 7 samples plus 1 negative control (NC) (N=8), four would be added to for n=12. This 12 would be multiplied by 1 for F-primer (and R-primer, double distilled water), and by 5 for Taq. Therefore, 12 µl of F-primer, 12 µl of R-primer, 12 µl double distilled water and 60 µl Taq would be added to the master mix.

Once all the laboratory equipment and notebook preparation was completed, the master mix was started by pipetting the calculated volume the double distilled water, F-primer and R-primer into a 1.5 ml Eppendorf tube. The sample was vortexed briefly to mix. Moving the master mix to the side for a moment, 2-3 µl of the DNA sample and 2-3 µl of double distilled water (for negative control) were pipetted (using either the 0.1 to 10 µl red top pipette or the 2 to 20 µl cream top pipette, the cream pipette was less precise) into the cut and labelled PCR plate. Taq was taken out of the freezer as briefly as possible (just enough time to liquefy). It was normally taken out when there were only 4 DNA samples left to pipette into PCR plates. Taq was then added to the master mixes which were vortexed again and 8 µl of master mix was pipetted into the DNA containing PCR plate wells. A plastic dropper pipette was used to add 1-2 drops of mineral oil into each sample containing well, ensuring that the tip always remained high above the wells to prevent contamination. Mineral oil was added to prevent sample evaporation, a problem Dr Tiddi with PCRs 1 to 5. Whenever PCR products were used, the pipette tip was placed at the bottom of the tube to avoid pipetting up oil. Eppendorf cap strips were then carefully and securely placed on top of the PCR plate, and the plate was taken to the PCR machine room.

Three different PCR machine were used: (1) MiniAmp (ThermoFisher Scientific), (2) C1000 Thermal Cycler (Bio Rad) and (3) GS1 (G-Storm). The MiniAmp was used most frequently, followed by C1000 then occasionally the G-storm was used. No difference is PCR product quality was observed. For all the microsatellites the thermal cyclers were set to the same base programmes. The samples were

placed into the PCR machine, the programme settings were checked, the lid was closed and the programme was started. The lid was heated to 105°C, and the machine was told the sample volume was 10 µl. For step 1, a 95°C hot start was performed for 1 minute. Next in step 2 the samples were denatured for 3 minutes at 95°C. For step 3, samples were annealed at their annealing temperature (52-62°C) for 30 seconds. Samples were elongated for 30 seconds at 72°C for step 4. Steps 2 to 4 were repeated in order 45 times. For step 5, a final extension was performed at 72°C for 15 minutes. The thermal cycler was then left cycling at 4°C for ∞. Depending on the lab work schedule, samples could be removed immediately after step 5 was finished, left at step 6 for a few hours, or left cycling at step 6 overnight (maximum 12 hours). Samples were then stored in the fridge until they were next needed.

As a way to increase PCR amplification success, for PCRs 76 to 90 (using extraction 7b samples), markers with an allele range surpassing 250 bp underwent a test to determine whether they amplified better with a 45 seconds vs 30 seconds elongation phase. Of these markers some amplified better with 30 seconds elongation while others ran better with a 45 second elongation. From PCR 91 (extraction 7c onwards), markers had the following elongation times, and were run grouped (or not) in the machine as follows (Table 2):

Table 2. Showing Marker Grouping, Elongation Times And Annealing Temperature PCR Machine Settings

Markers And PCR Machine Groupings[1]	*Elongation Time (Seconds)*	*T_a (Annealing Temperature) (°C)*[1]
D7S794	30	52
Ceb02	45	55
Ceb07	30	55
Ceb04	30	57
Ceb03, Ceb08, Ceb115	30	58
Ceb11, Ceb130	45	59
Ceb121	30	59
Ceb09, Ceb127, Ceb128	30	60
Ceb105	45	60
Ceb10	45	61
Ceb119	30	62

[1] *(Muniz and Vigilant, 2008, p. 403)*

Gels

Gels were numbered based on the PCR name (e.g. PCR 1) and the marker name (e.g. Ceb02). Fisher scientific brand 10X TBE buffer, was diluted with distilled water, to 0.5X TBE for shared lab use. In this study, this 0.5X was used to make agarose gels and was the liquid used for gel electrophoresis. To make one gel the following steps were followed and up to 4 gels were made at one time to go into the lab's 4 electrophoresis machines. 100 ml of 0.5X TBE was poured into a glass 250 ml flask. 2 grams (+/- 10) of ThermoFisher's agarose powder was weighed on a scale and then added to the TBE solution. The flask was gently swirled/rotated until the white agarose particles were seen evenly distributed throughout the TBE. The flask was placed in a microwaved at full power for 1.5-2 minutes. Samples were then checked to determine whether the solution was transparent and bubbling. If this was the case they were removed to cool for 10 minutes. If samples were not bubbling yet, or were bubbling but still opaque, samples were further microwaved being checked on every 1.5 minutes until transparent and bubbling. 1 flask microwaved alone tended to take 1.5-2 minutes to become bubbly and transparent. While when multiple (2-4) flasks were microwaved at once they took longer (up to 5 minutes) to reach this point. If during the microwaving process, too much of sample evaporated, a new mixture was made from scratch. Once done, all samples were left to cool for 10 minutes. During this time, gel trays were prepared by masking tape being used to seal their two non-walled sides. 2 Gel comb each having 16 wells (32 wells total per gel) were loaded onto the gel tray. Once flasks were cooled, 1 µl of ThermoFisher's SybrSafe DNA Gel Stain was carefully pipetted into the flask, which was then gently swirled to evenly distribute the dye. The flask's mixture was then poured into the gel tray with masking tape and combs, and left to set for 15 minutes. During this time, flasks were cleaned out and gel design was written out in the laboratory notebook. For PCRs 1 to 90, all PCR product samples were visualised on a gel. From PCR 91 (using extraction 7c individuals), only 3 to 4 samples plus the NC per marker, were run through the gel.

In gel electrophoresis tanks, 0.5X TBE was added if necessary (when levels were low and the TBE had been recently changed). TBE tank liquid was changed at least a month. PCR products were removed from the fridge. After the 15 minutes, gels were checked to ensure that they were firm and if they were, combs were removed. 2-4 µl of Thermo Scientific's GeneRuler ready to use, 50 bp DNA ladder was pipetted into the first well of each row as well as the first well before a marker's PCR product. For example if PCR products from 2 markers (n=12 per marker), 2 ladders would be placed, 1 at the top of each row which were followed by PCR product. However, if PCR products from 3 markers were used (n=8 per marker), 4 ladders would be placed. 2 ladders would be placed at the top of each row as well as 2 ladders which were placed in wells before a new PCR product started. Following this, on the work table 3 µl of PCR product was pipetted into each well. 12-13 wells were used, as the gel tray for the Gel Doc EZ Imager could not fit the full size of the gel and 2-3 wells were cut off with a sharp ruler. Masking tape was then removed from the gel trays, the electrophoresis machine's lid was removed and placed to the side. Gels were gently placed in the gel electrophoresis machine, ensuring that they were fully submerged and placed firmly in the middle. The lid was carefully placed on and the machine was turned on and ran at 100 – 140 volts for 45 minutes. If machine was running for less than 100 volts, the sample was left for an hour. When cycle was started the electrophoresis was determined to be working if there was bubbling at electrical input areas and the ladder and PCR product was moving across the gel.

Once the 45 minutes were up, it was checked that the ladder and samples completely travelled the gel. If this was the case, the machine was turned off, the gel was removed, placed on an upside down tray and the bottom 2-3 wells were cut off and disposed. The gel was then placed into the tray of the Gel Doc EZ Imager and Image Lab software was run to visualise the gel. This image was checked, had it's contrast changed if necessary for better viewing, each well was labelled and PCR number and marker was noted. Furthermore, from January 2022 all gels were analysed and placed into an excel PCR tracking document with contained a traffic light system which denoted how well each PCR (per marker) worked. Red denoted that no sampled amplified, orange that less than half of

the samples amplified and green denoted that more than half of the samples amplified (Appendix B. PCR and Gel Tracker).

Sending Plates To Durham University's DBS Genomics Laboratory

63 plates were sent to Durham for Genotyping. Each sample was genotyped across 2 (sometimes 3) dilutions per plate. The negative control was only tested at one dilution, as it was only necessary to determine whether there was any contamination. The majority of plates were made single plex. Over the study dilutions of: 1:10 (1 µl PCR product: 9 µl of double distilled water), 1:20 (1 µl PCR product: 19 µl of double distilled water), 1:50 (1 µl PCR product: 49 µl of double distilled water) and 1:100 (1 µl PCR product: 99 µl of double distilled water) were used. Dilutions 10 (1:10) and 20 (1:20) were used in tandem with dilution 50 (1: 50) and/or 100 (1:100) when genotyping a new marker or a new marker combination (in the case of post-PCR multiplexes). Most of the plates were done with dilutions 50 and 100 per sample. Plates were designed and transcribed/stuck into lab books before the lab session in which they were made. To design the plate, a fully bordered table with 96 boxes (1 per well) was drawn or typed out and each row (A – H) and column (1 -12) was labelled. Individual ID was noted in blue at the top left corner, extraction number was noted in red in the top right corner, marker number was noted in green in the bottom left corner and dilution amount was noted in black in the bottom right corner of each box (Appendix C. Durham University Plate Design). Any changes to plate design that occurred during the making of the plate were noted. Full plates had all 96 wells filled whereas half plates only had odd numbered columns filled (e.g 1,3,5…etc.) (Appendix C. Durham University Plate Design).

Both single- and multi- channel pipettes were used in all steps of plate creation. Once the necessary laboratory equipment was cleaned and labelled, a dilution plate was made by pipetting the required amounts of double distilled water (for example 49 µl for dilution 50) into a clean and empty 96-well

PCR plate. 1 µl of PCR product was then carefully pipetted into each well and a double pipetting (in and out the pipette twice) mixing technique was used. This was the technique for preparing singleplex plates. For plates where a Post-PCR multiplex was applied, 1 µl of PCR product A and 1 µl of PCR product B was added to the double distilled water in the dilution plate. One less micron of the double distilled water was pipetted into the dilution plate (for example 48 µl for dilution 50). Plates 19, 39, 53-54,57-59 and 62-63, had post-PCR multiplexes. Post-PCR multiplexes consisted of two microsatellites ideally with the different dye colours Fam/Tet and Tamra and at least 40 bp apart. Markers were paired once they were at least 40 base pairs apart. Plate 19 contained the multiplex Ceb121-11. Plates with multiplexes between 39 to 63, had the following 4 pairing: (1) Ceb08-127, (2) Ceb07-105, (3) Ceb09-10 and (4) Ceb115-130. Plates 62-63, additionally had pairings of Ceb02-D7S794 and Ceb119-128.

The dilution plate was then placed in plate centrifuge (salad spinner) and spun to better mix the PCR product and double distilled water. 5 µl of diluted PCR product was then pipetted into a new 96-well PCR postage plate. Thermo Scientific Nunc Seals were stuck to the dilution and the postage plates ensuring that any air bubbles were removed. The postage plate was then wrapped in a few sheets of blue roll and carefully placed into a bubble lined labelled envelope. A fragment analysis requisition form detailing information on the plates contained in the envelope was also placed in the envelope with was then sealed. The plates were then posted via first class mail thorough the Kent Estates Mail Service. Dilution plates were placed in the fridge for storage. A GM plate form which detailed information on each well's contents was also completed and emailed to Durham University (dna.sequencer@durham.ac.uk). Some of the wells in Plates 42 to 63 ended up evaporating before reaching Durham. Plate 63 contained redone (5 µl from dilution plate) evaporated samples for plates 42 to 46, which did not evaporate again. However, any evaporation that occurred in the wells of plates 47-63 were not redone due to the time and financial constraints of the problem. This meant that some samples only had 1 dilution.

Geneious Analysis

2-3 days after postage plates were received at Durham University, they would email back an FSA file that was genotyped for Geneious. Sometimes, single wells were rerun and sent separately by researchers at Durham. This was to prevent disturbances of electrophoresis which can affect sizing accuracy. A singular FSA file would contain information on all 96 wells of a (full) plate and would be opened in Geneious. The first step in Geneious would be to manually edit the ladder until it was recognisable as GeneScan 500, by Geneious. This process involved deleting excess peaks, adding missing peaks and ensuring that peaks were properly spaced. This was one for all 96 entries of the FSA file. Once the ladder was set, wells containing a locus were selected and the locus information was inputted into Geneious. All microsatellites were diploid and tetranucleotides. Dr Tiddi initially inputted the locus ranges listed in Muniz and Vigilant (2008, p. 403) used for *Cebus capucinus*. As the initial ranges were based on: (1) another species and (2) specific alleles present in the 187 individuals studied (Muniz and Vigilant, 2008), Dr Tiddi decided to experiment and expand allele ranges. During this experimentation phase, locus ranges were expanded to include a new peak found in Geneious. Across the 16 microsatellites this lead to an average range expansion of 28.125 base pairs (range 2 to 82) (Table 3). Once MMS took over this project, the ranges established by Dr Tiddi during the experimental expansion phase were kept with no further range modification occurring. Locus bins were automatically assigned by Geneious. As new plates were genotyped, new bins were assigned to new believable peaks (asymmetric, height matching established peaks for the plate, and seen in other individuals). When MMS took over the project, across all loci, bin labels and ranges were edited to: (1) ensure alleles were spaced 4bps apart and (2) standardise allele profiles between MMS and Dr Tiddi. Locus colour was also inputted into the locus information section. There were problems with Geneious reading loci which were FL with green Tet dye, as peaks often did no show up when the Tet setting was chosen. However, peaks showed up when blue Fam dye setting was chosen. Therefore, all Loci with Tet dye were read as Fam in Geneious (Table 3).

Table 3. Showing the Locus Information Inputted Into Geneious

LOCUS NAME[1]	NUMBER OF PEAKS	REPEAT UNIT NUMBER (BP)	REPEAT MOTIF[1]	ORIGINAL ALLELE RANGE (BP)[1]	ALLELE RANGE IN GENEIOUS (BP)	BIN SETS	DYE COLOUR
CEB02	2	4	$[TCTA]_9$	225-233	210-265	209.10 -> 211.49bp "210" 212.53 -> 215.64bp "214" 224.44 -> 227.68bp "226" 228.71 -> 231.81bp "230" 232.58 -> 235.68bp "234" 236.20 -> 239.55bp "238" 240.10 -> 243.80bp "242" 244.70 -> 247.53bp "246" 252.50 -> 255.40bp "254" 256.30 -> 259.90bp "258"	Fam
CEB03	2	4	$[GATA]_{11}$	177-201	146-235	154.48 -> 157.57bp "156" 158.23 -> 162.37bp "160" 170.50 -> 173.80bp "172" 190.40 -> 193.70bp "192" 194.60 -> 197.80bp "196" 198.70 -> 201.80bp "200" 202.50 -> 205.70bp "204" 210.50 -> 214.10bp "212" 215.30 -> 218.40bp "216" 223.80 -> 227.10bp "226" 228.00 -> 231.40bp "230"	Tet (read as **Fam)**
CEB04	2	4	$[TCTA]_{14}$	174-198	170-211	170.10 -> 173.30bp "172" 174.10 -> 177.60bp "176" 178.10 -> 181.30bp "180" 182.10 -> 185.30bp "184" 186.10 -> 189.80bp "188"	Tet (read as **Fam)**
CEB07	2	4	$[AGAT]_{10}$	119-135	123-173	123.00 -> 127.00bp "125" 127.30 -> 130.40bp "129" 131.10 -> 134.50bp "133" 135.00 -> 139.00bp "137" 139.20 -> 142.30bp "141" 143.20 -> 146.30bp "145" 146.40 -> 149.74bp "149" 159.00 -> 162.90bp "161" 171.00 -> 173.80bp "173"	Fam
CEB08	2	4	$[TAGA]_{12}$	161-189	155-190	155.00 -> 156.10bp "153" 156.50 -> 159.30bp "157" 159.50 -> 162.60bp "161" 163.70 -> 166.60bp "165" 167.60 -> 170.80bp "169" 171.30 -> 174.70bp "173" 175.30 -> 178.90bp "177" 179.10 -> 182.90bp "181" 183.10 -> 186.60bp "185" 187.40 -> 190.00bp "189"	Tamra
CEB09	2	4	$[ATCT]_{10}$	153-189	142-199	141.00 -> 143.76bp "142" 144.50 -> 147.40bp "146" 148.20 -> 151.30bp "150" 152.11 -> 155.47bp "154" 156.54 -> 159.35bp "158" 168.20 -> 171.40bp "170" 176.40 -> 179.40bp "178" 179.90 -> 183.30bp "182" 184.50 -> 188.30bp "186" 188.60 -> 191.50bp "190" 191.80 -> 196.20bp "194" 196.46 -> 199.30bp "198"	Fam
CEB10	2	4	$[AGAT]_{13}$	238-250	230-263	236.30 -> 239.50bp "238" 240.40 -> 243.60bp "242" 244.40 -> 247.30bp "246" 248.40 -> 252.50bp "250" 252.70 -> 256.20bp "254" 256.40 -> 259.50bp "258" 260.10 -> 263.30bp "262"	Tamra

CEB105	2	4	$[TAGA]_{10}$	236-244	210-252	220.20 -> 223.30bp "222" 224.10 -> 227.70bp "226" 228.45 -> 231.65bp "230" 232.20 -> 235.60bp "234" 236.45 -> 239.65bp "238" 240.20 -> 243.60bp "242" 244.45 -> 247.65bp "246" 248.45 -> 251.65bp "250"	Tamra
CEB11	2	4	$[TCTA]_{11}$	223-251	200-257	210.30 -> 213.40bp "212" 226.20 -> 229.70bp "228" 230.60 -> 233.80bp "232"	Tet (read as **Fam)**
CEB115	2	4	$[ATCT]_{11}$	122-134	120-140	125.20 -> 128.50bp "127" 129.40 -> 132.60bp "131" 133.80 -> 136.90bp "135"	Fam
CEB119	2	4	$[GATA]_{12}$	229-257	229-270	244.60 -> 248.50bp "247" 253.70 -> 256.20bp "255" 256.40 -> 260.40bp "259" 261.30 -> 264.40bp "263" 265.60 -> 268.50bp "267"	Tet (read as **Fam)**
CEB121	2	4	$[CTAT]_{10}$	140-184	116-200	116.00 -> 121.40bp "120" 121.70 -> 125.90bp "124" 126.30 -> 129.50bp "128" 130.30 -> 133.70bp "132" 134.60 -> 137.90bp "136" 142.00 -> 145.90bp "144" 150.30 -> 153.30bp "152" 154.10 -> 157.40bp "156" 157.90 -> 162.00bp "160" 162.50 -> 165.50bp "164" 170.30 -> 173.50bp "172" 174.60 -> 177.90bp "176" 182.20 -> 185.60bp "184" 194.10 -> 197.40bp "196" 198.10 -> 201.10bp "200"	Fam
CEB127	2	4	$[TATC]_{9}$	243-255	232-266	237.22 -> 240.51bp "239" 249.36 -> 252.85bp "251" 253.65 -> 256.68bp "255" 257.68 -> 260.45bp "259" 261.65 -> 264.85bp "263"	Fam
CEB128	2	4	$[CTAT]_{10}$	190-206	175-199	176.60 -> 180.70bp "179"	Fam
CEB130	2	4	$[ATCT]_{9}$	182-218	182-300	192.10 -> 195.60bp "194" 208.40 -> 211.70bp "210" 244.40 -> 247.20bp "246" 252.70 -> 255.50bp "254" 256.50 -> 259.30bp "258" 264.20 -> 267.90bp "266" 268.40 -> 272.20bp "270" 272.40 -> 276.00bp "274" 276.70 -> 279.80bp "278" 292.50 -> 295.90bp "294"	Tamra
D7S794	2	4	[GATA]	133-145	110-155	120.90 -> 123.90bp "122" 124.30 -> 127.90bp "126" 136.30 -> 140.00bp "138" 140.70 -> 143.60bp "142" 143.70 -> 147.40bp "146"	Tamra

[1](MUNIZ AND VIGILANT, 2008, P. 403)

Once Locus Information was established, and applied, Geneious assigned peaks were checked. Excel sheets were made for each locus per plate (Dr Tiddi) (Appendix D. Post-Geneious Excel Outputs) or for each locus per PCR ran (MMS) (Appendix D. Post-Geneious Excel Outputs). These documents contained: individual ID, plate number, plate comments, dilution, Geneious ID, Allele 1 for Locus,

Allele 2 for locus and Geneious comments (Appendix D. Post-Geneious Excel Outputs). The negative control was the first sample checked to determine if there had been contamination during the PCR process. If there were no peaks in the NC, the rest of the samples were then checked. If there were peaks in the NC, the peaks were then evaluated and it was decided whether these peaks were 'true' alleles. Peaks were discounted if they were too short and their height was either less than 500 RFU (Relative Fluorescence Units), or they were much shorter than the rest of the peaks in the plate (for example more peak height is approximately 25,000 RFU and the discounted peak is only 1,000 RFU). Peaks were also discounted on the basis of their shape, I.E they were discounted if they were (too) symmetrical or evenly curved. 'True' alleles have asymmetrical peaks. In the NC, peaks were also discounted if they occurred outside of bin sets as they were more likely to be a stutter band, and/or occurred at ranges that was not seen in the rest of the PCR/Plate. Any discounted peaks in the NC was still noted in the excel document, with these peaks being checked for in the rest of the contaminated PCR. Due to time and financial constraints, plates were unable to be redone in the lab. This was despite some plates being contaminated and many plates having peaks with very high RFU (up to 40,000 RFU) than usual (around 1,000 RFU). therefore, a procedure was developed for plates with NC contamination. For any 'true' alleles in the NC, the length (bp) and height (RFU) was noted. For the rest of the samples in that group, any peaks at the same length (bp) that were the same height (RFU) or less were discounted and not labelled. If a peak was present at the same length (bp) but was much taller (RFU), was accepted. In addition, any peaks at different lengths were accepted, once they were determined to be true alleles.

Each sample was checked to ensure that Geneious had correctly assigned peaks. In addition, to the height, shape, position criteria mentioned above, some first peaks were discounted. First peaks which were more than a third of the size of the second peak (for example first peak was 1,000 RFU while the second peak was 25,000 RFU). First peaks which were shorter and only 4bp apart from second peaks were closely evaluated to determine whether they were a stutter band or 'true' allele. In some cases more than 2 peaks were present. Firstly, each peak was evaluated to determine

whether it was a ‘true’ allele, and discounted if they were not. If there were still more than 2 peaks present, then all peaks were noted, and none were assigned. Any incorrectly assigned peaks were deleted and if there were any missed ‘true’ alleles, these were assigned. Any peaks which MMS/Dr Tiddi were unsure about were noted, reviewed again and if not resolved on a repeat viewing, they were discussed in meeting with Professor Jim Groombridge (and Dr Tiddi if MMS had the queries), where a group decision was made in the allele designation. Results from samples with multiple dilutions were also checked and compared, with any discrepancies being noted and all the individual’s dilutions were italicized in excel. Allele profiles were written as follows: any samples which had empty locus ranges were designated ‘no peaks’, for heterozygous samples each allele was written once i.e. 169/181, and for homozygous allele was written twice, for example 157/157. It was during this stage that Ceb128 was determined to be monomorphic and genotyping of these samples was abandoned.

Every sample was checked in Geneious at least 2 times before being added to the genotyping table. The genotyping table contained the following columns: individual ID, individual number, extract number, type of repeats, number of repeats, allele 1 for locus, allele 2 for locus, Geneious code and comments (Appendix E. Genotyping Table). The genotyping table was the excel document, where all the allele profiles were brought together. Once excel documents, for individual plates had been checked twice, one dilution (usually 50) from individual per marker was added to the table. If the profiles from the multiple dilutions did not match, firstly both samples and ladder assignments were rechecked for errors in Geneious. If there was still a mismatch, both allele profiles were noted in the genotyping document. In cases where one dilution had peaks while the other did not have peaks, the peaked allele profile was added to the document with the no peaked dilution being noted. In addition, plates with NC contamination were given a light blue background and the contaminated peak was noted. Samples with more than 2 clear peaks were given a light orange background, with peaks present being noted (Appendix E. Genotyping Table).

After the genotyping table was completed, a new excel document was created. In this document repeats from a microsatellite for an individual were compared. A traffic light system was developed. Green signified that the majority of repeats have the same allele profile for the locus. Red signified that the repeats have mismatching alleles (orange was originally used to denote similar alleles but was decided to denote these samples as red). In the final genotyping table, these red values and any no peaks designations were zeroed and given a grey background. Black signified that allele profile was only present once and the other repeat(s) had no peaks designation (Appendix E. Genotyping Table). For any mismatches, all repeats were checked ensuring that there were no mistakes in genotyping and ladder attribution. It was at this stage that standardization of the methods and locus information documents between Dr Tiddi and MMS was performed. At this stage, any queries MMS had regarding peak designations were discussed and finalised with the help of Professor Groombridge and Dr Tiddi. A final check was performed after initial Cervus analysis which revealed many mismatches between known mother and infant pairings. In this check all repeats across the 15 microsatellites across mother-offspring groupings were compared and once again checked for errors. It was at this point that it was realised that the same individual (Omar/Mora) had been sampled and genotyped twice. For Cervus analysis Omar's (OMA22) genotype was used for this individual.

Data Analysis

Summary Statistics And Error Checking

The final genotyping table was error checked through the excel add-ins Genalex and Microsatellite Toolkit. Microsatellite Toolkit was used to determine whether any alleles had been wrongly typed, and highlighted large gaps between alleles. Both Genalex and Microsatellite Toolkit were useful in identifying falsely called alleles that were only 2bps (and not 4bp) apart. Along with Cervus, Genalex and Microsatellite Toolkit also produced summary statistic tables. These tables provided a per locus breakdown of the total number of alleles, alleles present, counts and frequency (as a percentage) of each allele. Information on observed and expected heterozygosity, Shannon's Information Index (Genalex) and Polymorphic Information Content (PIC) (Microsatellite Toolkit and Cervus) was also available. Results from all three software were compared to ensure that each software was producing similar and reliable results. All three software produced the same results for alleles present, observed and expected heterozygosity. Microsatellite Toolkit and Cervus produced the same results for the PIC. Genalex and Cervus produced the same number of alleles and individuals typed per locus. Genalex also determined the number of effective alleles and percentage of missing data. Cervus additionally provided statistics on null allele frequencies and tested whether the markers were in Hardy-Weinburg equilibrium.

Table 4. Showing The Combined Summary Statistics Outputs Generated From Genalex, Cervus And Microsatellite Toolkit

Locus	N[1,2]	N_A[1,2]	N_E[1]	Missing Data (%)[1]	H_O[1,2,3]	H_E[1a,2,3]	PIC[2,3]	HW[2]	NAF[2]
Ceb02	59	5	2.286	15	0.407	0.567	0.520	NS	0.1520[4]
Ceb03	64	5	4.421	8	0.672	0.780	0.737	NS	0.0706[5]
Ceb04	64	5	1.627	8	0.406	0.388	0.352	ND	-0.0011
Ceb07	60	10	2.996	14	0.633	0.672	0.637	NS	0.0184
Ceb08	57	7	4.857	18	0.702	0.801	0.763	ND	0.0636[5]
Ceb09	66	7	3.065	6[b]	0.758	0.679	0.614	NS	-0.0658
Ceb10	51	4	2.384	27	0.353	0.586	0.520	NS	0.2434[4]
Ceb105	63	7	3.244	10	0.524	0.697	0.633	NS	0.1344[4]
Ceb11	63	2	1.364	10	0.222	0.269	0.231	ND	0.0917[5]
Ceb115	63	3	2.096	10	0.492	0.527	0.409	NS	0.0312
Ceb119	50	8	2.894	28	0.660	0.661	0.621	NS	-0.0192
Ceb121	41	18	13.448	41	0.488	0.937	0.921	ND	0.3050[4]
Ceb127	61	2	1.378	12	0.230	0.276	0.237	ND	0.0886[5]
Ceb130	60	7	4.016	14	0.750	0.757	0.716	NS	0.0009
D7S794	65	5	2.870	7	0.600	0.657	0.588	NS	0.0421

N: Number of individuals typed, N_A: Number of Different Alleles, N_E: Number of Effective Alleles = 1 / (Sum pi^2) (Where pi is the frequency of the ith allele for the population & Sum pi^2 is the sum of the squared population allele frequencies.), Missing Data: based on number of 0s/ non called alleles for locus, H_O: Observed Heterozygosity, H_E: Expected Heterozygosity, PIC: Polymorphic Information Content, HW: Hardy-Weinberg equilibrium test, NAF: Null allele frequency

[1] Genalex
[a] Genalex's uHe Measurement = Unbiased Expected Heterozygosity = (2N / (2N-1)) * He
[b] MMS' calculation as Genalex did not calculate for Ceb09
[2] Cervus
[3] Microsatellite Toolkit
[4] NAFs ≤ 0.10
[5] NAFs ≤ 0.05

Cervus And Paternity Analysis

There are many different methods to perform parentage analysis during microsatellite data (Jones *et al.*, 2010). The most commonly used methods include exclusion and likelihood (Marshall *et al.*, 1998; Slate, Marshall and Pemberton, 2000; Jones *et al.*, 2010). The exclusion method works by comparing offspring-potential parent genotypes, and excluding a potential parent based on any mismatches regardless of how much genetic information is provided (Marshall *et al.*, 1998; Slate, Marshall and Pemberton, 2000; Jones *et al.*, 2010). However, due to human error, mutations or null alleles and allelic dropout, true offspring-parent pairs could be excluded by this method. Therefore, the likelihood method has become much more commonly used (Marshall *et al.*, 1998; Slate, Marshall and Pemberton, 2000; Jones *et al.*, 2010). The likelihood method works by instead comparing the genotypes of the offspring and potential parents, potential parents are each assigned a probability

that the offspring is theirs. From this the most likely potential parent is assigned to the offspring (Marshall *et al.*, 1998; Slate, Marshall and Pemberton, 2000; Jones *et al.*, 2010; Walling *et al.*, 2010). The likelihood method can be applied in a myriad of different ways leading to the creation of different equations, software and more (Marshall *et al.*, 1998; Slate, Marshall and Pemberton, 2000; Jones *et al.*, 2010; Walling *et al.*, 2010).

For this study paternity analysis was performed by the Cervus 3.0 software, which infers parentage via Mendelian Inheritance from codominant microsatellite marker that have high PIC (Marshall *et al.*, 1998; Kalinowski, Taper and Marshall, 2007, 2010). Cervus uses the likelihood method based on equations by Marshall *et al.* (1998; Kalinowski, Taper and Marshall, 2007, 2010), to create the LOD and DELTA scores necessary to assign parentage. LOD is the log of the overall likelihood ratio and expresses the probability that a candidate parent is related to the offspring. As Cervus focuses on comparing the two most likely parents, sometimes LOD scores are not enough to determine the most likely father. Because of this Cervus also uses a DELTA (Δ) score, which are derived from the LOD, and calculates the difference between two positive LOD scores of the most likely parents (Marshall *et al.*, 1998; Kalinowski, Taper and Marshall, 2007, 2010). Furthermore, as well as assigning parents to infants, Cervus also uses two statistical confidences, strict (*) at 95% and relaxed (+) at 80%, to provide users with the certainty of the assignment (Marshall *et al.*, 1998; Kalinowski, Taper and Marshall, 2007, 2010). Cervus was chosen over other software like Colony, for its ease of use due to the great depth of background information in its help guide. On the one hand, Cervus' main focus is determining parentage. On the other hand, Colony starts by analysing data for sibling relationships, then creates familial groups, and then finally runs parentage analysis (Walling *et al.*, 2010). On top of this, Colony can also rely on behavioural data to make assignments which has the potential to bias assignments, for example, towards certain males (e.g alpha males). Finally, Colony has a more inaccessible interface and tends to be more conservative in parentage assignment than Cervus (Marshall *et al.*, 1998; Walling *et al.*, 2010).

Mother ID was known through behavioural observations, and paternity was tested by running all the group males who were present during the mating season of the infant's conception. In preparation of Cervus analysis, the genotyping file had to be converted from excel (.xlsx) to text file (.csv) (Appendix E. Genotyping Table). In addition, an offspring file was created in excel then converted to text file. It contained the following information: Offspring ID, Mother ID, Sex, Age Class, Birth Date, Birth Season, Group and Candidate Fathers (Appendix F: Cervus Offspring File). Males present in the group in the mating season (year) preceding an offspring's birth, were assigned as candidate fathers. Of the 70 individuals sampled, there was birth data: including known birth date/season, known mother ID and known potential fathers for 39 individuals spanning from 2006 to 2013 (Appendix F: Cervus Offspring File). However, due to missing genetic samples from 5 potential fathers and 2 known mothers, 12 of these individuals were excluded from paternity analysis. 8 individuals from Guenon with 1 missing potential father (CHA77) were kept in the Offspring file, as for these individuals the mother was known and only 1 of the 2 potential fathers had missing samples. Therefore, in total 27 infants were run through Cervus for paternity analysis (Appendix F: Cervus Offspring File).

For Cervus to run parentage assignment markers must be codominant from autosomal chromosomes of diploid species. in addition, the markers must not have any (low to moderate are tolerated) null alleles and need to be in Hardy-Weinburg and linkage equilibriums. Cervus calculates parentage assignment over three steps. In the first step, allele frequency analysis, Cervus analyses the loci via genotyping table and produces outputs detailing information of each loci (

Table 4). Particularly important outputs were the information detailing the test of Hardy-Weinburg Equilibrium, as if any loci were not in equilibrium (significant) they could not be used. Another important output was the Null Allele Frequency (NAF) per locus, as loci with high NAF affect Cervus' ability to assign parentage. The next step in Cervus is to run a simulation. Cervus simulations run by using the allele frequency data as well as inputted parameters such as the number of offspring, number and proportion of candidate fathers sampled, the numbers of loci typed and mistyped, the error likelihood and the minimum of typed loci for an individual to be included in analysis (

Table 5). The simulation is run to determine a critical DELTA, which determines the values for strict and relaxed confidence in the parentage analysis outputs..

Table 5. Showing the simulation parameters inputted for parentage analysis with 11 loci and used in statistical analysis

Input	
Number of offspring:	10000
Number of candidate fathers:	10
Proportion of candidate fathers sampled:	0.8462
Proportion of loci typed:	0.87400000
Proportion of loci mistyped:	0.01000000
Error rate in likelihood calculations:	0.01000000
Minimum number of typed loci:	6
Output	
Confidence determined using:	Delta
Relaxed confidence level:	80.00%
Strict confidence level:	95.00%

Parentage analysis is the third and final step of the Cervus programme. For this step, Cervus uses information from: the genotyping, offspring, allele frequency and simulation files to assign parentage to offspring. Two parentage analysis outputs are generated. The first section of the first output file relates to parentage assignment results. A table is created statistics for three sections: (1) Assignments based on comparisons between the candidate father and offspring where the known mother is not considered (2) Assignments based on comparisons between the candidate father and offspring where the mother is unknown/not tested (3) Assignments based on comparisons between the candidate father, known mother and offspring. These three sections each have a breakdown of observed vs assignments at strict and relaxed confidences, unassigned infants and a total of offspring tested. The next sections display the numbers of offspring, known mothers and candidate fathers tested, exclusions of individuals who are not typed at enough loci and missing individuals (according to Cervus). Cervus also outlines known mother-offspring mismatches, null alleles, locus and overall error rates. The second output file is an excel sheet that has the breakdowns of paternity assignment for each offspring. This file contains Offspring and Known Mother IDs, exclusion probabilities, a breakdown of loci: an individual's number, number compared and number of mismatches, most likely father, LOD and DELTA scores, Pair and Trio Confidences (-, + or *).

Statistical Analysis

To test objective 1 and whether rank predicts paternity success, an excel data file was created for use in IBM SPSS Statistics for Windows Version 28 (IBM Corp, 2021) was created. This excel file included the following data: Group, Year, Rank, Male ID, Offspring Sired (N Vs Y), Offspring ID, Total Offspring Sired (Per Group & Year), Number Of Offspring Sired (Per Group & Year) (0 Vs 1, SRP29 With 2 Kids In 1 Year (2013) Entered Twice) and Proportion Of Offspring Sired (Per Group + Year). Fieldwork data provided the information of group, year and rank (see

Behavioural Sampling). The paternity assignment data was transformed by MMS for statistical tests to be performed (Table 6). In SPSS, a Generalized Linear Mixed Model (GLMM) was run using a binary logistic regression (Serpico sired 2 infants in 2013 and the second infant was edited to be sired in 2014 for the model to run). The model was run with following parameters: Subject was Male ID, Repeated Measures were Year, Target was N. of Offspring Sired, Fixed Effects was Rank with an Intercept and Random Effects with Intercept. The Save Fields Field for Predicted Values was also selected so that the model's predictions of which males were siring offspring could be checked (Appendix I: SPSS). This model was run through a couple of times to determine whether rank could be used as a predictor and to gain a better understanding of how the model and SPSS works. In order to understand whether the findings of the GLMM could be apply to capuchins as a whole, the data was then split into two groups: (1) Macuco (model data), and (2) Guenon and Spot (validation data). A GLMM was fit using the Macuco data. The predictive power of the model was then tested using the validation dataset. The model was trying to approximate the wider Iguazú population.

Table 6. Showing SPSS Input For Objective 1: Whether Rank Predicts Paternity Success

Group	Year	Rank	Male ID	Off Sired	Off ID	Total Off Sire	N Of Off Sired	Prop. of Off Sired
Guenon	2006	1	MAR16	N		0	0	0
Guenon	2006	2	CHA77	N		0	0	0
Guenon	2007	1	MAR16	N		0	0	0
Guenon	2007	2	CHA77	N		0	0	0
Guenon	2008	1	MAR16	N		0	0	0
Guenon	2008	2	CHA77	N		0	0	0
Guenon	2009	1	MAR16	Y	ALE36	1	1	1
Guenon	2009	2	CHA77	N		1	0	0
Guenon	2010	1	MAR16	Y	ISI54	1	1	1
Guenon	2010	2	CHA77	N		1	0	0
Macuco	2010	1	ERN9	Y	ESB46	1	1	1
Macuco	2010	2	SRP29	N		1	0	0
Macuco	2010	3	SER27	N		1	0	0
Macuco	2010	4	JUS80	N		1	0	0
Macuco	2010	5	TTO32	N		1	0	0
Macuco	2010	6	EDU7	N		1	0	0
Macuco	2011	1	ERN9	Y	ZIS75	2	1	0.5
Macuco	2011	2	SRP29	N		2	0	0
Macuco	2011	3	SER27	Y	LEN57	2	1	0.5
Macuco	2011	4	JUS80	N		2	0	0
Macuco	2011	5	TTO32	N		2	0	0
Macuco	2011	6	EDU7	N		2	0	0
Macuco	2012	1	ERN9	Y	MAT58	2	1	0.5
Macuco	2012	2	SRP29	N		2	0	0
Macuco	2012	3	SER27	Y	PIE25	2	1	0.5
Macuco	2012	4	TTO32	N		2	0	0
Macuco	2012	5	EDU7	N		2	0	0
Macuco	2013	1	ERN9	Y	JC56	3	1	0.33
Macuco	2013	2	SER27	N		3	0	0
Macuco	2013	3	EDU7	N		3	0	0
Macuco	2013	4	SRP29	Y	SIM70	3	1	0.33
Macuco	2013	4	SRP29	Y	CAB40	3	1	0.33
Macuco	2013	5	TTO32	N		3	0	0
Macuco	2014	1	ERN9	N		0	0	0
Macuco	2014	2	SER27	N		0	0	0
Macuco	2014	3	EDU7	N		0	0	0
Spot	2011	1	TRU31	Y	SYR73	1	1	1
Spot	2011	2	RIN26	N		1	0	0
Spot	2012	1	TRU31	N		1	0	0
Spot	2012	2	RIN26	N		1	0	0
Spot	2012	3	TET74	Y	HUR52	1	1	1
Spot	2013	1	TRU31	N		0	0	0
Spot	2013	2	TET74	N		0	0	0
Spot	2013	3	RIN26	N		0	0	0
Spot	2014	1	TRU31	N		0	0	0
Spot	2014	2	RIN26	N		0	0	0
Spot	2014	3	TET74	N		0	0	0

The Priority of Access Model (objective 2) was tested in a myriad of ways. Firstly, additions were made to the dataset created for objective 1 (Table 6). The columns of Group-Year, Number of Males, Number of Ovulating Females, Mother ID and Expected Success (ES) were added. Proportion Of Offspring Sired (Per Group + Year) was renamed to Observed Success (OS) (Table 8). 2014 entries for Macuco and Spot were removed as although male rank was known, infant birth occurred after the study period ended so no faecal and hair samples were collected. SRP29's (Serpico, Macuco group) two 2013 entries were reduced to one. The number of ovulating females was determined by Dr Tiddi by using data from an earlier study (Tiddi, Wheeler and Heistermann, 2015). Dr Tiddi determined ovulating females per group-year by using females with hormonally verified peri-ovulatory periods. For 5 years across the 3 groups (Macuco 2010, Macuco 2012, Guenon 2011, Spot 2011 and Spot 2012), Dr Tiddi looked at the degree of female synchrony using females with hormonally verified conceptive cycles. These cycles were noted and the timings of cycles were compared between females to create a measure of female synchrony (Table 7). Cycle overlap values were then used along with male rank to come up with an expected success measure. Following Dubuc *et al.* (2011), depending on the numbers of proceptive females present, males were assigned an equal share of paternity based on rank. Alphas were assigned a proportion of paternity first and were followed by betas who were followed by gammas etc. For example, if there was one proceptive female, only alphas were assigned paternity (1.00), and all other males were assigned no share of paternity. If there were two proceptive females, alphas and betas (0.50 each) were both assigned shares of paternity. Once each cycle was calculated, each male's sum of shares was calculated. This sum of shares per male was divided by the total sum of shares to create a proportion value for paternity success (Table 7, Appendix H: Female Synchrony And Expected Success). This proportion value was created as a measure for rank based male expected success. For Guenon, expected success values calculated in 2011, was used for all the years that were tested. For Macuco and Spot, where two years of expected success values existed, for each rank expected success values were averaged. These averages of expected success (as a proportion) were used for Macuco and Spot individuals for all the years tested.

In SPSS, this dataset (Table 6) was run using family of the GLMM where the target was in Poisson distribution with a log link function (Loglinear setting in SPSS). The PoA GLMM was run with most of the same Inputs as the rank GLMM, the fixed effects was changed to ES (with intercept) and group (with intercept) was added to some runs. The target remained the number of offspring sired (0, 1, 2), instead of OS, due to Poisson's based count distribution and the fact that none of the distributions supported a target with a proportion based value (Appendix I: SPSS). The data was run once including 2014 data to ensure that removing this data was not what caused the model to not fit adequately. Due to the fact that the GLMM model was not fitting the data satisfactorily (high standard errors and large confidence intervals), other methods of statistical analysis were employed to gain a better understanding of the relationship between ES and OS. For this analysis, the data was split to be organised and analysed on a year by year basis, with all years (2010- 2013) having 8 entries. Guenon's entries from 2006 to 2009 were excluded as the sample sizes for these years was too small (there were only two entries). Following this Kendall's tau correlations, a nonparametric method good for small sample sizes, were run comparing expected success and observed success variables. Scatter plots with lines of best fit with ES on the x-axis and OS on the y-axis, were also created. A two-tailed paired sample T-Test, which is designed to work with very small samples ($n \leq 30$), was also run over the years comparing ES with OS. The variables adhered to the assumptions of the T-Test, the variables were: (1) continuous, (2) approximately normally distributed and (3) did not contain outliers. In addition, each observation was be independent of each other.

Table 8. Showing SPSS Data Input For Objective 2: Testing The Priority Of Access Model

Group	Year	Group-Year	N of males	N of ovulating fem	Male Rank	Male ID	Total Off Sired	N of Offsp	Offspring ID	Mother ID	Expected	Observed
Guenon	2006	GUE2006	2	1	1	MAR16	0	0			1	0
Guenon	2006	GUE2006	2	1	2	CHA77	0	0			0	0
Guenon	2007	GUE2007	2	2	1	MAR16	0	0			1	0
Guenon	2007	GUE2007	2	2	2	CHA77	0	0			0	0
Guenon	2008	GUE2008	2	2	1	MAR16	0	0			1	0
Guenon	2008	GUE2008	2	2	2	CHA77	0	0			0	0
Guenon	2009	GUE2009	2	3	1	MAR16	1	1	ALE36	ELE8	1	1
Guenon	2009	GUE2009	2	3	2	CHA77	1	0			0	0
Guenon	2010	GUE2010	2	1	1	MAR16	1	1	ISI54	LIL14	1	1
Guenon	2010	GUE2010	2	1	2	CHA77	1	0			0	0
Macuco	2010	MAC2010	6	6	1	ERN9	1	1	ESB46	SOL28	0.6	1
Macuco	2010	MAC2010	6	6	2	SRP29	1	0			0.33	0
Macuco	2010	MAC2010	6	6	3	SER27	1	0			0.07	0
Macuco	2010	MAC2010	6	6	4	JUS80	1	0			0	0
Macuco	2010	MAC2010	6	6	5	TTO32	1	0			0	0
Macuco	2010	MAC2010	6	6	6	EDU7	1	0			0	0
Macuco	2011	MAC2011	6	4	1	ERN9	2	1	ZIS75	EST10	0.6	0.5
Macuco	2011	MAC2011	6	4	2	SRP29	2	0			0.33	0
Macuco	2011	MAC2011	6	4	3	SER27	2	1	LEN57	THE30	0.07	0.5
Macuco	2011	MAC2011	6	4	4	JUS80	2	0			0	0
Macuco	2011	MAC2011	6	4	5	TTO32	2	0			0	0
Macuco	2011	MAC2011	6	4	6	EDU7	2	0			0	0
Macuco	2012	MAC2012	5	7	1	ERN9	2	1	MAT58	MAW17	0.6	0.5
Macuco	2012	MAC2012	5	7	2	SRP29	2	0			0.33	0
Macuco	2012	MAC2012	5	7	3	SER27	2	1	PIE25	YOL35	0.07	0.5
Macuco	2012	MAC2012	5	7	4	TTO32	2	0			0	0
Macuco	2012	MAC2012	5	7	5	EDU7	2	0			0	0
Macuco	2013	MAC2013	5	4	1	ERN9	3	1	JC56	COR5	0.6	0.33
Macuco	2013	MAC2013	5	4	2	SER27	3	0			0.33	0
Macuco	2013	MAC2013	5	4	3	EDU7	3	0			0.07	0
Macuco	2013	MAC2013	5	4	4	SRP29	3	2	SIM70, CAB40	ING12, THE30	0	0.67
Macuco	2013	MAC2013	5	4	5	TTO32	3	0			0	0
Spot	2011	SPO2011	2	2	1	TRU31	1	1	SYR73	JOS13	0.75	1
Spot	2011	SPO2011	2	2	2	RIN26	1	0			0.25	0
Spot	2012	SPO2012	3	2	1	TRU31	1	0			0.75	0
Spot	2012	SPO2012	3	2	2	RIN26	1	0			0.25	0
Spot	2012	SPO2012	3	2	3	TET74	1	1	HUR52	EVA11	0	1
Spot	2013	SPO2013	3	2	1	TRU31	0	0			0.75	0
Spot	2013	SPO2013	3	2	2	TET74	0	0			0.25	0
Spot	2013	SPO2013	3	2	3	RIN26	0	0			0	0

Table 7. Showing The Proceptive Females Data And Expected Success Calculations For Macuco 2010

Cycles Mac 2010	Date	Sub	Number of proceptive females	ERN	SRP	SER	JUS	TIT	EDU		
1	07/06/10	THE	2	0.5	0.5	0	0	0	0		
2	11/06/10	YOL	1	1	0	0	0	0	0		
3	24/06/10	EVA	3	0.33	0.33	0.33	0	0	0		
4	08/07/10	THE	2	0.5	0.5	0	0	0	0		
5	15/07/10	CLA	2	0.5	0.5	0	0	0	0		
6	24/07/10	CHI	1	1	0	0	0	0	0		
7	06/08/10	THE	2	0.5	0.5	0	0	0	0		
				4.33	**2.33**	**0.33**	**0**	**0**	**0**	**6.99**	**EXP (SUC**
				0.62	0.33	0.05	0	0	0	1	PROP. ES

Objective 3 was based on predicting whether non-alpha males would commit infanticide or not based on their overall paternity success (Research Aims And Hypotheses). This was tested across groups through the years both by rank and by male ID. This analysis was all performed in Microsoft Excel (Version 2302). For the Van Schaik equation, two **P** values were calculated as percentages from

alpha OS: (1) P by group-year which was a converted OS/Proportion measure and (2) P by group which was the average success of an alpha over the years tested. Two **p** values, which were based on non-alpha males, were also calculated as percentages: (1) p group-year-rank which matches OS/Proportion of Infants Sired, and (2) p-rank which was calculated based on the fraction of overall success for each rank. For example, there were 12 infants assigned fathers and 7 (58.33%) of them were assigned to alphas, while 3 (25%) of them were assigned to gammas. The data was then placed into Pivot Tables where calculations could be made based on different aspects of the data, for example rank and group across the years. It was through the Pivot Tables that the adapted Van Schaik equation was run, using the following formula in excel:

$$= \frac{20.4-14.1}{20.4} *' P\ group\ year' -' p\ group\ year\ rank' \ldots \textit{Equation 6}$$

For further t-analysis, two Pivot Tables were prepared. The first was prepared where the subject was group-rank while in the second, the subject was Male ID (see *Table 9* for formulas calculated for analysis).

Table 9. Showing Excel Formulas Used In Infanticide Objective 3

Function Name	Excel Formula	Notes
Sample Mean (%)	=AVERAGEIF(B6:I6,"<>0")	Average of values (more or less than 0) across the years
Squared Deviations (%)	=IF(E6=0,"",(E6-$K6)^2)	If Subject (Male-ID or Group-Rank) value is 0, leave blank, Year value for Male ID minus Sample Mean squared
Count (N)	=COUNTIF(M6:T6,">-100")	Count if Squared Deviation values are numeric
Degrees of freedom	=IF(V6>1,V6-1,1)	Degrees of freedom appropriate for the t-test
Sample Variance (%)	=SUM(M6:T6)/W6	Sum of Squared Deviations divided by degrees of freedom
Sample Standard Deviation (%) (Appendix J. Statistical Analysis)	=SQRT(X6)	Square root of variance
Population Mean (%)	Set to 0 (Null)	Assumed under Van Schaik (equation is 0)
Standard Error (%)	=Y6/SQRT(V6)	Sample standard deviation divided by the square root of the count
T (N)	=IF(ISERROR((K6-Z6)/AA6),0,(K6-Z6)/AA6)	This calculates the t value for the data
T. Confidence (%)	=IF(ISERROR(CONFIDENCE.T((1-AC4),Y7,V7)),0,CONFIDENCE.T((1-AC4),Y7,V7))	T. Confidence was estimated using the Student's t distribution. It was used to produce the 95% Confidence Interval for the population by adding or subtracting the T. Confidence from the Sample Mean.
Lower Bound Population Van Schaik (%)	=K6-AC6	Sample Mean minus T. Confidence
Upper Bound Population Van Schaik (%)	=K6+AC6	Sample Mean plus T. Confidence
95% Population Confidence Interval (CI) (range: e.g [-0.7, 1.2])	="["&ROUND(AD6,1)&","&ROUND(AE6,1)&"]"	Lower Bound Rounded to 1 decimal place (dp), Upper Bound rounded to 1 dp
Commit Infanticide	=IF(AD6>0,"commit infanticide", IF(AE6<0,"wont commit infanticide", "inconclusive"))	If Lower bound (and upper) is greater than 0 marked as commits infanticide, if upper bound (and lower) is less than 0 is marked as won't commit infanticide, otherwise marked as inconclusive

Results

Genotyping And Paternity Analysis

Of the 70 individuals genotyped across the 15 microsatellites, 49 individuals (70%) were successfully genotyped at least 13 loci (13 individuals at 15 loci, 20 at 14 loci & 17 individuals at 13). The remaining 21 (30%) individuals were genotyped at 12 to 5 loci (6 individuals at 12 loci, 5 at 11, 3 at 10, 1 at 9, 2 at 8, 1 at 7, 2 at 6 and 1 at 5). The 4 individuals who were genotyped at less than half of the markers were: EST10 (Estela) at 7 markers, SOL28 (Sol) and RIC66 (Ricky) at 6 loci and finally MAV59 (Maverick) at 5 loci (Appendix A. Study Subjects & Appendix E. Genotyping Table). In the initial Cervus analysis, which was run on 39 offspring, only 4 offspring were assigned fathers (all at * confidence) (Appendix G. Cervus Outputs). This low assignment rate was thought to be due to the high (98) numbers of mismatches between known mother-offspring pairs and the high average error rate of 0.51 across all 15 microsatellites (Appendix G. Cervus Outputs). Due to the high mismatch rate, it was decided to re-check the genotyping table for errors, this time comparing mother-offspring pairs (Appendix E. Genotyping Table). This reduced the mismatches to 38 and the average error rate across the markers to 0.27 (Appendix G. Cervus Outputs). However, assignment rate remained low, with only 6 assignments across 5 (of 39) offspring being made (5 at relaxed confidence, and 1 at strict confidence) (Appendix G. Cervus Outputs). As the assignment rate still remained low, the Cervus inputs were experimented with. Loci with high error rates, like Ceb121 (was also removed singularly), Ceb02, D7S794, Ceb105, Ceb127, Ceb10, were removed in groups of 2 to 5 loci at a time. This lead to a maximum of 11 infants having assigned fathers. At this point, the offspring file was rechecked against the field data by Dr Tiddi, and the new offspring file with 27 individuals was created. In addition, MMS started excluding loci with NAFs of over 0.05 (as recommended by the Cervus help guide) to improve assignment rate. A total of 8 (of 15) loci had NAFs of 0.05 or more (Table 4), and so two approaches were tested. In the first approach, the 4 loci with NAFs of 0.10 or more were excluded, leaving 11 loci to be included in paternity analysis. In the

second approach, all 8 loci with NAFs of 0.05 or more were excluded, leaving 7 loci to be tested. When 11 loci were tested 16 assignments across 12 infants at relaxed or strict confidence were gotten (Table 10, Appendix G. Cervus Outputs: Paternity Analysis Output For The 11 Loci). Of these 16 assignments, 11 were assigned with strict confidence and 5 were assigned with relaxed confidence. In addition, 9 assignments were made based on pair (candidate father-offspring) confidence and 7 were made based on trio confidence (known mother-candidate father-offspring). 4 offspring were assigned at both pair and trio confidence. The 7 loci test got 21 assignments across 14 infants (Table 11, Appendix G. Cervus Outputs: Paternity Analysis Output For The 7 Loci). However, 4 of these assignments were at – (most likely father not assigned) level. 12 assignments were at relaxed confidence while 5 were at strict confidence. In total 12 infants had assignments at a minimum of relaxed confidence. In addition, 11 assignments were made based on pair confidence and 10 were made based on trio confidence. 4 offspring were assigned at both pair and trio confidence. 2 offspring also had one – assignment at pair level and a +/* second assignment at trio level. The 11 loci test was ultimately used as this test has greater power based on the use of more loci. In addition this test had no – assignments, as well as more strict confidence assignments and the same amount of relaxed confidence assignments.

Table 10. Showing the paternity assignments for the 11 loci test

Offspring	Loci typed	Mother	Loci typed	Pair loci com	Pair loci mismatch	Pair LOD score	Candidate fa	Loci typed	Pair loci com	Pair loci mis	Pair LOD sc	Pair Delta	Pair con	Trio loci com	Trio loci mis	Trio LOD sc	Trio Delta	Trio conf
ALE36	11	ELE8	7	7	0	2.11E+00	MAR16	9	9	0	1.19E+00	1.19E+00	+	9	0	3.65E+00	3.65E+00	*
ISI54	10	LIL14	11	10	0	-8.08E-01	MAR16	9	8	1	-8.86E-01	0.00E+00		8	1	1.85E+00	1.85E+00	*
LEN57	8	THE30	11	8	1	-1.36E+00	SER27	10	7	0	2.70E+00	0.00E+00		7	1	2.56E+00	1.88E+00	*
ZIS75	11	EST10	0	0	0	0.00E+00	ERN9	11	11	0	4.17E+00	4.17E+00	*	0	0	0.00E+00	0.00E+00	
PIE25	10	YOL35	10	9	0	1.32E+00	SER27	10	9	0	2.81E+00	7.06E-01	+	9	1	-7.43E-01	0.00E+00	
JC56	11	COR5	10	10	1	-8.93E-01	ERN9	11	11	0	2.51E+00	5.87E-01	+	11	2	2.37E-01	2.37E-01	+
SIM70	10	ING12	11	10	0	3.06E+00	SRP29	11	10	0	2.46E+00	2.46E+00	*	10	2	-4.51E+00	0.00E+00	
ESB46	11	SOL28	6	6	2	-7.35E+00	ERN9	11	11	0	7.24E-01	7.24E-01	+	11	2	1.24E+00	1.24E+00	*
CAB40	10	THE30	11	10	0	3.79E+00	SRP29	11	10	0	2.57E+00	0.00E+00		10	0	4.86E+00	3.13E+00	*
HUR52	7	EVA11	9	6	4	-1.53E+01	TET74	10	7	0	2.36E+00	2.36E+00	*	7	4	1.46E+00	1.46E+00	*
MAT58	11	MAW17	11	11	1	-3.50E+00	ERN9	11	11	0	3.58E+00	3.04E+00	*	11	3	-1.68E+00	0.00E+00	
SYR73	9	JOS13	11	9	1	-1.85E+00	TRU31	7	5	0	3.09E+00	3.09E+00	*	5	1	-5.42E-02	0.00E+00	

Table 11. Showing paternity assignments for the 7 loci test

Offspring	Loci typed	Mother	Loci typed	Pair loci com	Pair loci mismatch	Pair LOD score	Candidate	Loci typed	Pair loci com	Pair loci mis	Pair LOD sc	Pair Delta	Pair con	Trio loci com	Trio loci mis	Trio LOD sc	Trio Delta	Trio con
HOR51	7	ELE8	3	3	0	-1.50E+00	MAR16	5	5	0	1.78E+00	1.78E+00	+	5	0	3.72E+00	3.72E+00	*
ALE36	7	ELE8	3	3	0	2.24E-01	MAR16	5	5	0	-7.22E-02	0.00E+00		5	0	1.76E+00	1.76E+00	+
ISI54	7	LIL14	7	7	0	-3.62E-01	MAR16	5	5	0	2.69E+00	2.69E+00	*	5	0	5.36E+00	5.36E+00	*
OMA22	7	ING12	7	7	0	1.93E+00	ERN9	7	7	0	7.47E-01	7.47E-01	-	7	1	-1.61E+00	0.00E+00	
LEN57	5	THE30	7	5	1	-3.42E+00	SER27	6	4	0	7.31E-01	0.00E+00		4	1	6.76E-01	6.76E-01	+
ZIS75	7	EST10	0	0	0	0.00E+00	SRP29	7	7	0	2.51E+00	2.67E-01	-	0	0	0.00E+00	0.00E+00	
PIE25	6	YOL35	6	5	0	4.18E-02	SER27	6	5	0	1.54E+00	7.06E-01	-	5	0	2.00E+00	2.00E+00	+
JC56	7	COR5	6	6	0	9.15E-01	ERN9	7	7	0	2.36E+00	1.99E+00	+	7	1	-5.17E-01	0.00E+00	
ESB46	7	SOL28	4	4	1	-3.60E+00	TTO32	7	7	0	1.06E+00	9.25E-01	+	7	1	2.45E+00	1.49E+00	+
CAB40	6	THE30	7	6	0	1.52E+00	SRP29	7	6	0	2.20E+00	4.32E-01	-	6	0	3.31E+00	3.31E+00	*
HUR52	3	EVA11	5	2	2	-7.83E+00	TET74	6	3	0	1.24E+00	1.24E+00	+	3	2	9.37E-01	9.37E-01	+
MAT58	7	MAW17	7	7	0	2.61E-01	EDU7	7	7	1	-6.88E-01	0.00E+00		7	1	1.28E-01	1.28E-01	+
DAT43	5	DAN6	7	5	1	-2.45E+00	TRU31	5	4	0	2.04E+00	2.04E+00	+	4	0	2.72E+00	2.72E+00	*
SYR73	6	JOS13	7	6	1	-2.69E+00	TRU31	5	4	0	1.58E+00	1.58E+00	+	4	1	-1.05E+00	0.00E+00	

Statistical Analysis

Overview of the Assignments

Of the 11 total potential fathers tested between 2006 and 2014, only six (54.54%) were assigned offspring. These males were MAR16 (Marcelo) who was alpha of the Guenon group and assigned two infants between 2009 and 2010. From the Macuco group, ERN9 (Ernesto), the alpha was assigned to four infants between 2010 and 2013. Also in the Macuco group was SER27 (Sergio) who when he was a gamma, sired two infants between 2011 and 2012. The final male from Macuco, who sired infants was SRP29 (Serpico) and was the only male to sire two infants in one year (2013) and the only delta to sire. In Spot, the alpha male TRU31 (Trucho) sired one infant in 2011, while the gamma male TET74 (Tetro) sired one infant in 2012. Therefore, across the groups alphas were slightly more successful, siring seven (58.33%) of 12 infants, while non-alpha males sired five (41.67%) of 12 infants (Table 12). In a further breakdown of individual rank success, gammas sired three (25.00%) of the 12 offspring while deltas sired two (16.67%) of the 12 offspring (Table 13).

Table 12. Showing paternity assignments detailing Offspring ID, Birth Year, Father ID and Rank And Group

Offspring ID	Birth Season (Year)	Father ID	Father Rank	Group
ALE36	2009	MAR16	1	GUE
ISI54	2010	MAR16	1	GUE
ESB46	2010	ERN9	1	MAC
ZIS75	2011	ERN9	1	MAC
MAT58	2012	ERN9	1	MAC
JC56	2013	ERN9	1	MAC
LEN57	2011	SER27	3	MAC
PIE25	2012	SER27	3	MAC
SIM70	2013	SRP29	4	MAC
CAB40	2013	SRP29	4	MAC
SYR73	2011	TRU31	1	SPO
HUR52	2012	TET74	3	SPO

Table 13. Showing paternity success based on group and rank (alpha vs non-alpha)

Group	Total Assigned	Assigned To Alpha (N)	Assigned To Alpha (%)	Assigned To Non-Alpha (N)	Assigned To Non-Alpha (%)
GUE	2	2	100.00	0	0.00
MAC	8	4	50.00	4	50.00
SPO	2	1	50.00	1	50.00
Totals:	12	7	58.33	5	41.66

Table 14. Showing paternity assignments by group and male ranks (1-6)

Group	Total Assigned	Assigned To Rank 1		Assigned To Rank 2		Assigned To Rank 3		Assigned To Rank 4		Assigned To Rank 5		Assigned To Rank 6	
		(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)	(N)	(%)
GUE	2	2	100.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
MAC	8	4	50.00	0	0.00	2	25.00	2	25.00	0	0.00	0	0.00
SPO	2	1	50.00	0	0.00	1	50.00	0	0.00	0	0.00	0	0.00
Totals	**12**	**7**	**58.33**	**0**	**0.00**	**3**	**25.00**	**2**	**16.67**	**0**	**0.00**	**0**	**0.00**

During the years of study for each group, alpha males had rank stability and kept alpha tenure. In Guenon, there was only one other male CHA77 (Chango), who remained a beta male throughout the study. In Macuco and Spot, non-alpha rank assignment shifted between males throughout the years,

with males being promoted or demoted in rank and/or dispersing during the study (Table 15 & Table 16). For example, in Spot 2012, RIN26 (Ringo) was beta while Tetro was gamma, and they reversed roles for 2013 (Table 15). Interestingly, Tetro became a beta the year (2013) the year after he sired HUR52 (Hurlio). Sergio similarly sired LEN57 (Lenin) in 2011 and PIE25 (Pietro) in 2012 while he was still a gamma, but became a beta in 2013. As there are only two cases, this evidence is still only anecdotal, however, this is an interesting potential male reproductive strategy for further study. This is because both Tetro and Sergio, first ascended ranks in the years in which they had vulnerable infants. Serpico on the other hand, had two kids when he was at his lowest rank (4), and then disappeared the following year. Another unexpected observation is the fact that beta males did not sire any offspring, especially since all 3 non-alpha males who sired offspring were betas at other points during the study.

Table 15. Showing male rank changes for Spot 2011-2014

YEAR RANK	2011	RANK CHANGE 2011-2012	2012	RANK CHANGE 2012- 2013	2013	RANK CHANGE 2013- 2014	2014
1	TRU31	Same	TRU31	Same	TRU31	Same	TRU31
2	RIN26	Same	RIN26	To Rank 3	TET74	To Rank 3	RIN26
3			TET74	To Rank 2	RIN26	To Rank 2	TET74

Table 16. Showing male rank changes for Macuco 2010-2014

YEAR RANK	2010	RANK CHANGE 2010-2012	2012	RANK CHANGE 2012- 2013	2013	RANK CHANGE 2013- 2014	2014
1	ERN9	Same	ERN9	Same	ERN9	Same	ERN9
2	SRP29	Same	SRP29	To Rank 2	SER27	Same	SER27
3	SER27	Same	SER27	To Rank 3	EDU7	Same	EDU7
4	JUS80	Disappeared	TTO32	To Rank 4	SRP29	Disappeared	
5	TTO32	To Rank 4	EDU7	To Rank 5	TTO32	Disappeared	
6	EDU7	To Rank 5					

Objective 1: Rank And Paternity

Preliminary Tests

Preliminary analysis of running the whole dataset through a GLMM (binary logistic) determined that rank significantly predicts paternity success (F= 6641864.832, df1= 5, df2= 41, p= 0.000) (Appendix J. Statistical Analysis: Preliminary Tests). Furthermore, the fitting model process indicated the correct number of offspring 70.2% of the time. The diagonals cells of the table which show what the model correctly predicted. The top left cell, shows 0s (no offspring) being accurately predicted 65.7% of the time and bottom right cell, shows 1s (having offspring) being predicted right 83.3% of the time (Table 17). The antidiagonals, represent (1): 0s wrongly predicted as 1s (bottom left: 16.7%) and (2) 1s wrongly predicted as 0s (top right: 34.3%) (Table 17). In addition, studying the Predicted Value column (Table 18), it is clear that the model predicted that both the alphas and gammas of all groups would sire offspring over all the years. Interestingly, considering both Sergio and Serpico sired 2 offspring each, the model did not predict that deltas would sire offspring. In contrast to what was expected, the model also did not predict betas as siring offspring. This is peculiar given the ordinality of rank and the assumption that the model should be predicting paternity success based on rank, with higher ranking males siring more offspring.

Table 17. GLMM SPSS Classification Table indicating model fit for paternity

Classification
Overall Percent Correct = 70.2%[a]

Observed		Predicted 0	Predicted 1
0	Count	23	12
	% within Observed	65.7%	34.3%
1	Count	2	10
	% within Observed	16.7%	83.3%

a. Target: N Of Offspring Sired (per group + year)

Table 18. With the Predicted Value column showing how the GLMM assigned paternity

Group	Year	Rank	MaleID	N Off Sired	Predicted Value
Guenon	2006	1	MAR16	0	1
Guenon	2006	2	CHA77	0	0
Guenon	2007	1	MAR16	0	1
Guenon	2007	2	CHA77	0	0
Guenon	2008	1	MAR16	0	1
Guenon	2008	2	CHA77	0	0
Guenon	2009	1	MAR16	1	1
Guenon	2009	2	CHA77	0	0
Guenon	2010	1	MAR16	1	1
Guenon	2010	2	CHA77	0	0
Macuco	2010	1	ERN9	1	1
Macuco	2010	2	SRP29	0	0
Macuco	2010	3	SER27	0	1
Macuco	2010	4	JUS80	0	0
Macuco	2010	5	TTO32	0	0
Macuco	2010	6	EDU7	0	0
Macuco	2011	1	ERN9	1	1
Macuco	2011	2	SRP29	0	0
Macuco	2011	3	SER27	1	1
Macuco	2011	4	JUS80	0	0
Macuco	2011	5	TTO32	0	0
Macuco	2011	6	EDU7	0	0
Macuco	2012	1	ERN9	1	1
Macuco	2012	2	SRP29	0	0
Macuco	2012	3	SER27	1	1
Macuco	2012	4	TTO32	0	0
Macuco	2012	5	EDU7	0	0
Macuco	2013	1	ERN9	1	1
Macuco	2013	2	SER27	0	0
Macuco	2013	3	EDU7	0	1
Macuco	2013	4	SRP29	1	0
Macuco	2014	4	SRP29	1	0
Macuco	2013	5	TTO32	0	0
Macuco	2014	1	ERN9	0	1
Macuco	2014	2	SER27	0	0
Macuco	2014	3	EDU7	0	1
Spot	2011	1	TRU31	1	1
Spot	2011	2	RIN26	0	0
Spot	2012	1	TRU31	0	1
Spot	2012	2	RIN26	0	0
Spot	2012	3	TET74	1	1
Spot	2013	1	TRU31	0	1
Spot	2013	2	TET74	0	0
Spot	2013	3	RIN26	0	1
Spot	2014	1	TRU31	0	1
Spot	2014	2	RIN26	0	0
Spot	2014	3	TET74	0	1

Due to the lack of betas being predicted a sensitivity analysis was run on the data. The data was manipulated so that across the years betas in Spot and Macuco, were marked as having offspring. At first, one beta in the whole dataset was manipulated (Ringo in 2011 from Spot). Then progressively the data was manipulated so either (1) multiple different beta males over different years were added (e.g. from Spot Tetro in 2013 & Ringo from 2014) or (2) the same male as beta was manipulated over multiple years (e.g. Serpico in 2010, 2011 & 2012). However, the model was very insensitive to these additions, and continued to not predict the betas. It was only when all the betas in Macuco and Spot in all years (all betas model) were manipulated to have kids that the model recognised the betas. The fixed effects results for all betas mode was as follows: an F statistic of 1735832.188 (df1= 5, df2= 41 and p=0.000) (Appendix J. Statistical Analysis: Preliminary Tests). The classification table shows that the model correctly predicted paternity 59.6% of the time, with 0s correctly predicted 34.6% and 1s correctly predicted 90.5% of the time (Table 19). This model predicted that ranks 1 to 3 from all groups, would sire infants every year (Table 20).

Table 19. Showing Classification Table showing the beta sensitivity investigations

Classification
Overall Percent Correct = 59.6%[a]

Observed		Predicted 0	Predicted 1
0	Count	9	17
	% within Observed	34.6%	65.4%
1	Count	2	19
	% within Observed	9.5%	90.5%

a. Target: N Of Off Sired (per group + year)

Table 20. Showing predicted values for beta investigation with all betas

Group	Year	Rank	MaleID	N Off Sired	PredictedValue
Guenon	2006	1	MAR16	0	1
Guenon	2006	2	CHA77	0	1
Guenon	2007	1	MAR16	0	1
Guenon	2007	2	CHA77	0	1
Guenon	2008	1	MAR16	0	1
Guenon	2008	2	CHA77	0	1
Guenon	2009	1	MAR16	1	1
Guenon	2009	2	CHA77	0	1
Guenon	2010	1	MAR16	1	1
Guenon	2010	2	CHA77	0	1
Macuco	2010	1	ERN9	1	1
Macuco	2010	2	SRP29	1	1
Macuco	2010	3	SER27	0	1
Macuco	2010	4	JUS80	0	0
Macuco	2010	5	TTO32	0	0
Macuco	2010	6	EDU7	0	0
Macuco	2011	1	ERN9	1	1
Macuco	2011	2	SRP29	1	1
Macuco	2011	3	SER27	1	1
Macuco	2011	4	JUS80	0	0
Macuco	2011	5	TTO32	0	0
Macuco	2011	6	EDU7	0	0
Macuco	2012	1	ERN9	1	1
Macuco	2012	2	SRP29	1	1
Macuco	2012	3	SER27	1	1
Macuco	2012	4	TTO32	0	0
Macuco	2012	5	EDU7	0	0
Macuco	2013	1	ERN9	1	1
Macuco	2013	2	SER27	1	1
Macuco	2013	3	EDU7	0	1
Macuco	2013	4	SRP29	1	0
Macuco	2014	4	SRP29	1	0
Macuco	2013	5	TTO32	0	0
Macuco	2014	1	ERN9	0	1
Macuco	2014	2	SER27	1	1
Macuco	2014	3	EDU7	0	1
Spot	2011	1	TRU31	1	1
Spot	2011	2	RIN26	1	1
Spot	2012	1	TRU31	0	1
Spot	2012	2	RIN26	1	1
Spot	2012	3	TET74	1	1
Spot	2013	1	TRU31	0	1
Spot	2013	2	TET74	1	1
Spot	2013	3	RIN26	0	1
Spot	2014	1	TRU31	0	1
Spot	2014	2	RIN26	1	1
Spot	2014	3	TET74	0	1

Through the preliminary and sensitivity tests, a few things become evident. First that the sample size is too small causing the model to overfit. One consequence of overfitting could be that betas and deltas were not predicted at all. In addition, the predicted values, where males sire every year, in

contrast to the observed success, where males do not sire every year, highlight that observed success is lower than expected. Therefore, the model is highlighting that paternity success might be the result of more factors than just rank.

Testing The Data: Model And Validation Set

Once the preliminary tests were run, the data was split into two partitions, the first part is used in fitting the model and the second part is used to test that the model is predicting well. Macuco made up the model set while Guenon/Spot made up the validation test set. The aim of this method was meant to be a test of whether rank predicts paternity in the general *Sapajus nigritus* population. Using the model set, the GLMM found that rank was a very significant (p= 0.001, F= 21.956, df1=5, df2=20) (Appendix J. Statistical Analysis). Like in the preliminary test, the model fit indicates that alphas and gammas would sire infants every year (Table 18). This model was 76.9% accurate, with 0s accurately predicted 77.8% of the time while 1s were predicted accurately 75.0% (Table 21). SPSS's scoring wizard was then used to generate the predicted values the validation test set. This data was then moved to excel were expected and observed success was compared to create the classification table (Table 22). The GLMM based on the Macuco modelled set, predicted paternity in the Guenon/Spot validation set very well (Table 23). In only one instance did the model wrongly predict an offspring. This lead to an overall of 97.1% correct assignment, with 1s being correctly assigned in 100% of the cases and 0s being correctly assigned in 94.1% of the cases. Therefore, this model fit the validation set very well. Therefore, rank can be used to predict paternity (Objective 1). This suggests that the model would accurately predict paternity in the wider Iguazú population. However, due to the small sample size (small amount of both repeated measures: years and subjects: males), as well as the fact that the model was overfitting and had an inability to predict betas, these results must be interpreted with caution.

Table 21. Showing the Classification Table for Model fit test set (Objective 1)

Classification

Overall Percent Correct = 76.9%[a]

Observed		Predicted 0	Predicted 1
0	Count	14	4
	% within Observed	77.8%	22.2%
1	Count	2	6
	% within Observed	25.0%	75.0%

a. Target: N Of Offspring Sired (per group + year)

Table 22. Showing the validation set and how the classification table was calculated

Group	Year	Male ID	Off Sired	Offspring	Rank	Total Group	N of Off Sired	Predicted Value	Observed	Expected	O-E	Obs0=Exp0	Obs1=Exp1
Guenon	2006	MAR16	N		1	0	0	1	0%	0%	0%	1	0
Guenon	2006	CHA77	N		2	0	0	0	0%	0%	0%	1	0
Guenon	2007	MAR16	N		1	0	0	1	0%	0%	0%	1	0
Guenon	2007	CHA77	N		2	0	0	0	0%	0%	0%	1	0
Guenon	2008	MAR16	N		1	0	0	1	0%	0%	0%	1	0
Guenon	2008	CHA77	N		2	0	0	0	0%	0%	0%	1	0
Guenon	2009	MAR16	Y	ALE36	1	1	1	1	100%	100%	0%	0	1
Guenon	2009	CHA77	N		2	1	0	0	0%	0%	0%	1	0
Guenon	2010	MAR16	Y	ISI54	1	1	1	1	100%	100%	0%	0	1
Guenon	2010	CHA77	N		2	1	0	0	0%	0%	0%	1	0
Spot	2011	TRU31	Y	SYR73	1	1	1	1	100%	100%	0%	0	1
Spot	2011	RIN26	N		2	1	0	0	0%	0%	0%	1	0
Spot	2012	TRU31	N		1	1	0	1	0%	100%	-100%	0	0
Spot	2012	RIN26	N		2	1	0	0	0%	0%	0%	1	0
Spot	2012	TET74	Y	TET75	3	1	1	1	100%	100%	0%	0	1
Spot	2013	TRU31	N		1	0	0	1	0%	0%	0%	1	0
Spot	2013	TET74	N		2	0	0	0	0%	0%	0%	1	0
Spot	2013	RIN26	N		3	0	0	1	0%	0%	0%	1	0
Spot	2014	TRU31	N		1	0	0	1	0%	0%	0%	1	0
Spot	2014	RIN26	N		2	0	0	0	0%	0%	0%	1	0
Spot	2014	TET74	N		3	0	0	1	0%	0%	0%	1	0
								Count (0)	17	16	-1	16	4
								Count (1)	4	5			

Notes:
Observed and Expected (%) was determined by dividing the O/E value by Total Group Offspring Sired
Excel's Count If function was used to calculate the numbers of 0s and 1s

Table 23. Showing the Classification Table for Validation Set (Objective 1)

Classification

Overall Percent Correct = 97.1%[a]

Observed		Predicted 0	Predicted 1
0	Count	16	1
	% within Observed	94.1%	5.9%
1	Count	0	4
	% within Observed	0.0%	100.0%

a. Target: N Of Offspring Sired (per group + year)

Objective 2: PoA And Paternity

Poisson GLMM

Another GLMM type, Poisson for count data, was run comparing expected success to the number of offspring sired (a count variable which was a proxy for OS), did not fit satisfactorily. This is clearly indicated from the F statistic of 0.000 and 1.000 significance with df1= 1 and df2= 38 (Appendix J. Statistical Analysis: Objective 2: PoA and Paternity). Similarly looking at the Fixed Coefficient Outputs for expected success, the model is not significant (1.000), with a large Standard Error (20713.4569) and large confidence interval (-41933.057 to 41931.346) (

Table 24). A high significance suggests that the model indicates that there is a weak relationship between expected success and paternity success. In addition, the large error and CIs further undermine the validity of the fit.

Table 24. Showing the Fixed Coefficients Output for PoA GLMM

Fixed Coefficients[a]						
Model Term	Coefficient	Std. Error	t	Sig.	95% Confidence Interval	
					Lower	Upper
Expected Success	-.856	20713.4569	.000	1.000	-41933.057	41931.346
Probability distribution: Poisson Link function: Log[a]						
a. Target: N of Offspring Sired						

Graphs And Correlations

Based on the fact that the GLMM provided a poor fit to the data, further investigation were run in order to assess any relationship between ES and OS. Kendall's tau Correlations and Scatter plots were then produced on a year by year basis. The results of this analysis were highly variable over the years. Four years were tested each having 8 males (Appendix J. Statistical Analysis: Objective 2: PoA and Paternity). In 2010, two males sired offspring Ernesto (0.60, 1.00) and Marcelo (1.00, 1.00) (Figure 1). The Kendall's tau indicated that ES and OS were strongly positively associated with a correlation coefficient of 0.739 that was significant (two-tailed) at 0.033 with a small CI of 0.291 - 0.921 (Table 25). In the graph, the R^2 Linear value provides a basic measure of goodness of fit (distance of points from the line) and the larger the value, the better the fit. The slope equation serves as a proxy for the correlation coefficient. For the 2010 graph (Figure 1), the R^2 Linear value is 0.828 which indicates that the line of best fit is fitting well. In addition, the slope (Figure 1), indicates that there is a positive association between ES and OS, which corroborates the findings of the Kendall's tau correlations. Visually there seems to be points close to and at either side of the line.

Table 25. Showing Correlation Results for year 2010

Confidence Intervals of Kendall's tau_b[a]

	Kendall's tau_b	Significance (2-tailed)	95% Confidence Intervals (2-tailed)[b]	
			Lower	Upper
Expected Success - Observed Success	.739	.033	.291	.921

a. Year = 2010

b. Estimation is based on Fisher's r-to-z transformation.

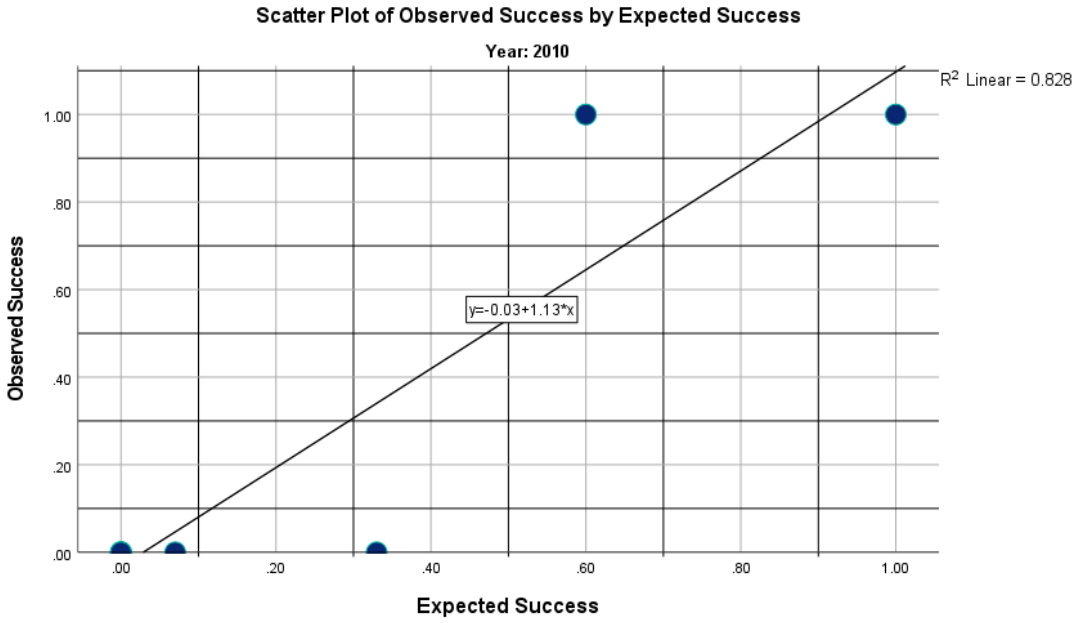


Figure 1. Showing ES vs OS for 2010

For 2011, three offspring were sired to: Ernesto (0.60, 0.50), Sergio (0.07, 0.50) and Trucho (0.75, 1.00) (**Error! Reference source not found.Error! Reference source not found.**). The ES and OS values were positively associated (Kendall's tau= 0.631) but were just above significance (0.053) and CI was quite small (0.094 – 0.883) (Table 26). In the graphs, it was less well fitting (R^2=0.572) and the slope (**Error! Reference source not found.Error! Reference source not found.**) showed a positive association between the variables.

Table 26. Showing Correlation Results for 2011

Confidence Intervals of Kendall's tau_b[a]

	Kendall's tau_b	Significance (2-tailed)	95% Confidence Intervals (2-tailed)[b]	
			Lower	Upper
Expected Success - Observed Success	.631	.053	.094	.883

a. Year = 2011

b. Estimation is based on Fisher's r-to-z transformation.

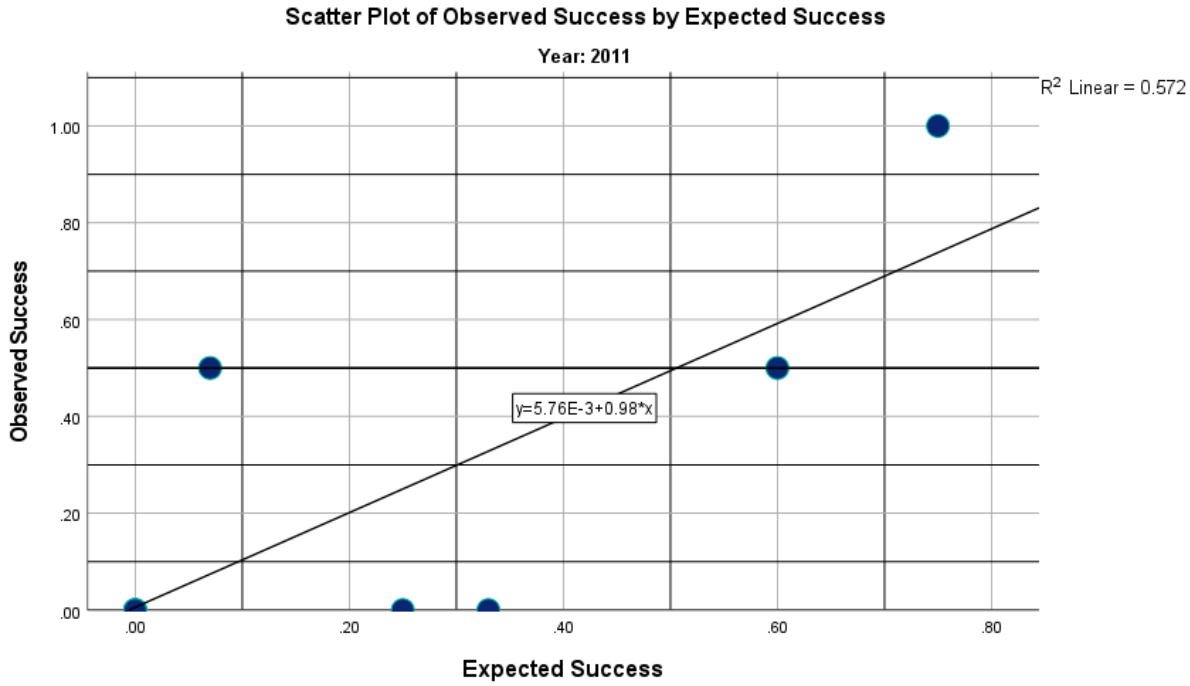


Figure 2. Showing ES vs OS for 2011

In 2012, three offspring were sired to: Ernesto (0.60, 0.50), Sergio (0.70 ,0.50) and Tetro (0.00, 1.00) who were well above the best fit line as outliers (Figure 3). The variables weakly associated (-0.146) (Table 27, Figure 3), nor significant (0.656) and had wide CI (-0.661 - 0.463). For the graph, the association was very low (0.045) and the slope (Figure 3) indicated almost no fit and a negative association.

Table 27. Showing Correlation Results for 2012

Confidence Intervals of Kendall's tau_b[a]

	Kendall's tau_b	Significance (2-tailed)	95% Confidence Intervals (2-tailed)[b]	
			Lower	Upper
Expected Success - Observed Success	-.146	.656	-.661	.463

a. Year = 2012

b. Estimation is based on Fisher's r-to-z transformation.

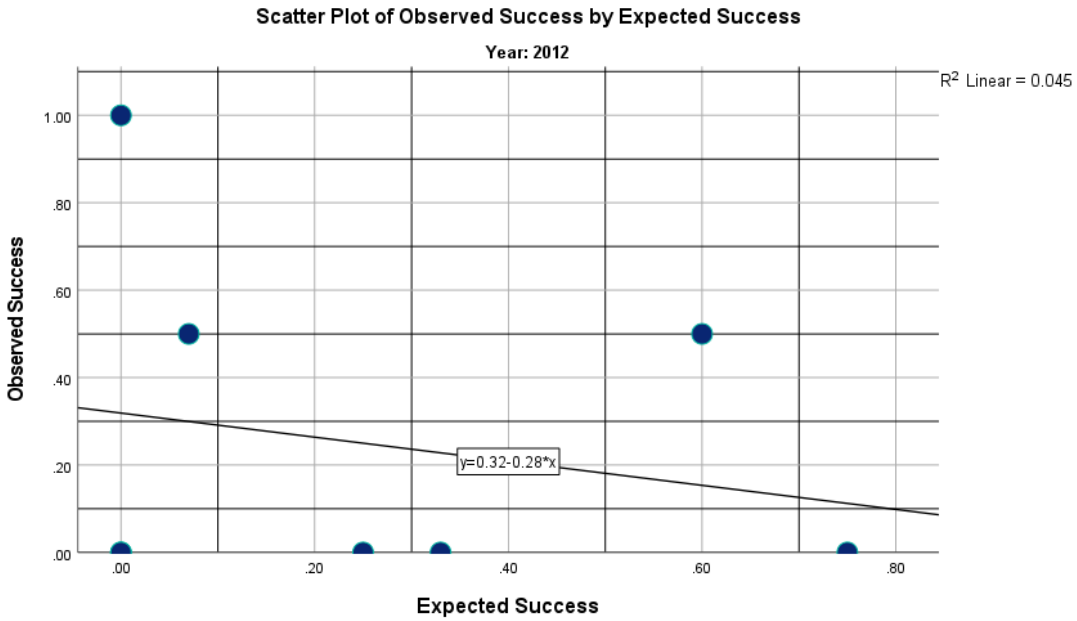


Figure 3. Showing ES vs OS for 2012

In 2013, two males sired infants: Ernesto (0.60, 0.33) and Serpico (0.00, 0.67), both of whom were outliers (Figure 4). ES and OS were neither significant (0.866) nor associated (-0.055) and had wide CI (-0.606 – 0.532) (Table 28). In Figure 4 there, a negligible R^2 value of 0.010 and the slope suggests a negative associated matched by the negative correlation coefficient which is difficult to interpret. Not surprisingly this can be seen in the graph where the points are far from the line.

Table 28. Showing Correlation Results for 2013

Confidence Intervals of Kendall's tau_b[a]

	Kendall's tau_b	Significance (2-tailed)	95% Confidence Intervals (2-tailed)[b]	
			Lower	Upper
Expected Success - Observed Success	-.055	.866	-.606	.532

a. Year = 2013

b. Estimation is based on Fisher's r-to-z transformation.

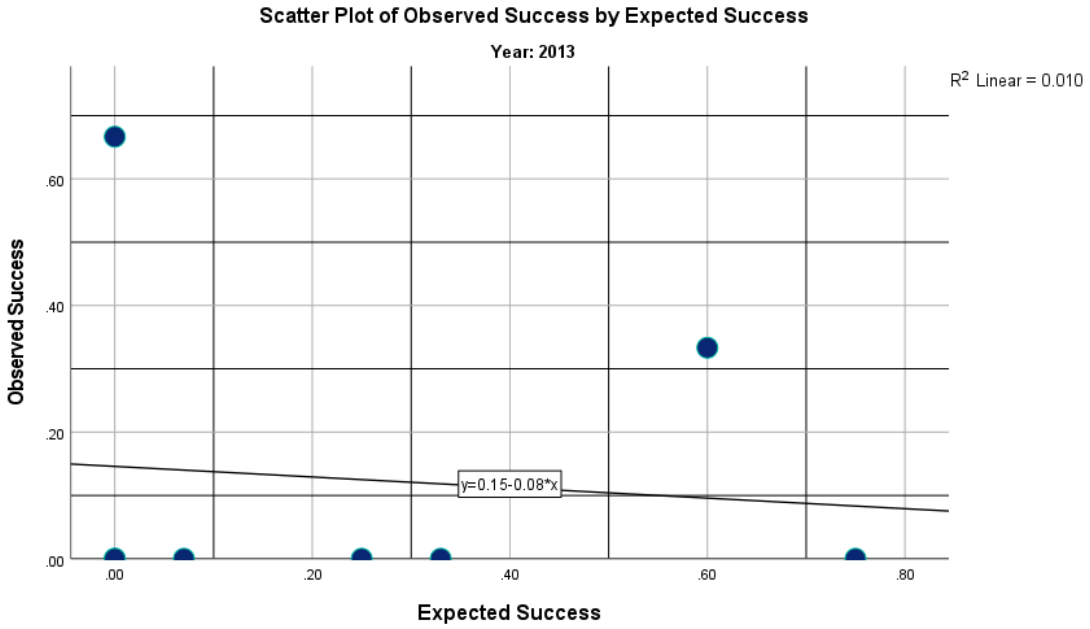


Figure 4. Showing ES vs OS for 2013

Overall trends in corelations greatly varied from year to year. Furthermore, the goodness of fit seemed to depend on which rank was siring infants. When only alphas sired (2010) there was a strong positive association between ES and OS. However, in 2013, when Serpico (a delta) sired more infants than the alphas a very weak negative association was seen instead. This variability explains why the GLMM provided such a poor fit. In addition, it highlights the paucity of data and how more males are needed for tests to be able to run and produce more conclusive results. Had there been beta males siring it would be expected that the trends would have been more stable and consistent from year to year.

Paired Sample T-Test

The final test of Objective 2, was a two tailed Paired Sample T-Test for each of the four years (2010 to 2014) to offset confounding effects of any negative and/or positive deviations. This T-Test was run to test the (Null Hypothesis) of whether ES=OS. In all years, 2010 (t= 0.000, Two-sided p=1.000) (Table 29), 2011 (t- -0.451, p = 0.666) (Table 30), 2012 (t= 0.000, p =1.000) (Table 31) and 2013 (t= 0.880, p= 0.408) (Table 32), t was very small and p was not significant indicating that ES is not statistically dissimilar to OS. This means that the PoA based calculation of ES (Dubuc *et al.*, 2011), worked relatively well and overall closely matched true paternity success.

Table 29. Showing Paired Sample T-Test for year 2010

Paired Samples Test[a]

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	Expected Success - Observed Success	.0000000000	.1977733190	.0699234275	-.165342632	.1653426324	.000	7	.500	1.000

a. Year = 2010

Table 30. Showing Paired Sample T-Test for year 2011

Paired Samples Test[a]

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	Expected Success - Observed Success	-.062500000	.3923464213	.1387154075	-.390509817	.2655098167	-.451	7	.333	.666

a. Year = 2011

Table 31. Showing Paired Sample T-Test for year 2012

Paired Samples Test[a]

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	Expected Success - Observed Success	.0000000000	.5249217629	.1855878691	-.438845576	.4388455760	.000	7	.500	1.000

a. Year = 2012

Table 32. Showing Paired Sample T-Test for year 2013

Paired Samples Test[a]

		Paired Differences					t	df	Significance	
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			One-Sided p	Two-Sided p
Pair 1	Expected Success - Observed Success	.1250000000	.4016671606	.1420107865	-.210802150	.4608021498	.880	7	.204	.408

a. Year = 2013

Objective 3: Infanticide

Exploring the results from the Van Schaik calculations for both Group-Rank and Male ID (both provided the same results- Appendix J. Statistical Analysis: Objective 3: Infanticide), a subject's tendency towards committing infanticide varied over the years (Table 33). In addition, for subjects who sired infants, in the years in which they sired no offspring, they were likely to commit infanticide. However, they would not be likely to commit infanticide in the years that they sired offspring. Based on the T calculations, Chango and Ringo (Lower bound= 0.3, Upper bound=0.3), Eduardo and Tito (0 - 0.3) were all likely to commit infanticide in all the years studied. This is because they had positive confidence intervals. Justo (-0.7 – 1.2), Sergio (-0.6 – 0.5) and Serpico (-0.6 – 0.6) were all inconclusive, as their intervals all ranged from positive to negative. While Tetro(-1 – (-1)) was not likely to commit infanticide, as this CI was entirely negative. Chango, Ringo and Tetro's results cannot be relied on for a population estimate as they were based on t calculations run on one entry (Table 34). Males which were marked has likely to commit infanticide or won't commit infanticide, provide evidence to support the alternative hypothesis (H1). While inconclusive males, provide evidence for the null, where the Van Schaik equation is equal to zero (Null).

In order to make more concrete conclusions, larger sample of males over a longer duration of time would be necessary. These calculations are all theoretical. No instances of infanticide were reported to MMS during the study period. In addition, a major stimulus for infanticide to occur, new alpha

takeover, did not occur and in fact alpha rank remained stable during the study period. However, it is still possible to conclude that in the years studied, females did not confuse paternity enough to prevent infanticide in the case of a male takeover. This is because, only Tetro who only had one entry was marked as not committing infanticide.

Table 33. Showing the Van Schaik Calculations for Male ID

Sum of Van Schaik	2006	2007	2008	2009	2010	2011	2012	2013	S_Mean
CHA77	0.0%	0.0%	0.0%	30.9%	30.9%	0.0%	0.0%	0.0%	**30.9%**
EDU7	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	10.3%	**18.0%**
JUS80	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	0.0%	0.0%	**23.2%**
RIN26	0.0%	0.0%	0.0%	0.0%	0.0%	30.9%	0.0%	0.0%	**30.9%**
SER27	0.0%	0.0%	0.0%	0.0%	30.9%	-34.6%	-34.6%	10.3%	**-7.0%**
SRP29	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	-56.4%	**1.3%**
TET74	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	-100.0%	0.0%	**-100.0%**
TTO32	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	10.3%	**18.0%**

Note: Red= will commit infanticide, green= won't commit infanticide

Table 34. Showing the T Calculations for Male ID

	Count	Degrees o	S_VAR	S_Std	P_Mean	S.E	t	T.Confide	Lower Bou	Upper Bou	95% POP_CI	Commit infanticide
CHA77	2	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3,0.3]	commit infanticide
EDU7	4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide
JUS80	2	1	1.2%	10.9%	0.0%	7.7%	3.00	98.1%	-74.9%	121.3%	[-0.7,1.2]	inconclusive
RIN26	1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3,0.3]	commit infanticide
SER27	4	3	10.8%	32.9%	0.0%	16.5%	- 0.42	52.4%	-59.4%	45.4%	[-0.6,0.5]	inconclusive
SRP29	4	3	15.3%	39.2%	0.0%	19.6%	0.07	62.3%	-61.0%	63.7%	[-0.6,0.6]	inconclusive
TET74	1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	-100.0%	-100.0%	[-1,-1]	wont commit infanticide
TTO32	4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide

Note: Purple= individuals with only 1 entry so t calculations less reliable, Red= will commit infanticide, green= won't commit infanticide, Refer to methods

Evaluations

Microsatellite Markers

Eighteen markers usable and informative in *Cebus capucinus* were originally presented (Muniz and Vigilant, 2008). In *Sapajus nigritus*, Ceb01 and Ceb120 did not amplify well and were excluded during the screening process. Ceb128 was excluded during genotyping as it was monomorphic. In addition, Ceb02, Ceb10, Ceb105 and Ceb121, had very high NAFs over 0.10 and were excluded from parentage analysis (Table 4). While Ceb03, Ceb08, Ceb11 and Ceb127, were not excluded in final parentage analysis but had high levels of NAFs (over 0.05). Of the remaining loci, Ceb07 was difficult to call in Geneious as often more than 2 peaks would be present in the locus range. In addition, Ceb04 and Ceb119 were FL green (Tet) but were analysed in blue dye (Fam), and it is possible this affected allele profiles. In only three markers, Ceb115, Ceb130 and D7S794, were there no problems. Overall, it was possible to use fifteen of these markers to genotype 70 individuals and assign 12 offspring paternity. Furthermore, these markers could have been more effective, if more repeats were performed. This is because some of the individuals, like MAW17 (Maw) (repeated 7 times), who were called at every marker, were repeated more than twice. Added to this, effectiveness of the markers could have also been increased by verifying homozygous alleles by performing at least 4 repeats, more if necessary. For heterozygous profiles, it would have been more effective if thee had been at least three repeats (Taberlet *et al.*, 1996; Van Belle, Estrada and Di Fiore, 2014).

Old DNA Samples

This project used (mainly) faecal and hair samples which were collected between 2010 and 2014. Extractions were carried out in 2019, 2021 with the majority occurring 2022. In 2019, the samples were between 5 and 9 years old. In 2021, the samples were between 7 and 11 years old, while in

2022 they were between 8 and 12 years old. This means that added to the original poor quality of DNA contained in the faecal samples, these samples were also relatively old, and the DNA in them may have degraded over time. This is likely due to the high degree of mismatching between known mother-offspring pairs as well as the fact that 8 loci had NAFs over 0.05. Furthermore, 6 samples which had two repeats performed on them had 7-10 markers with missing data. This is suggests that these 6 samples were not genotyped well due to degraded DNA in the faecal sample. Furthermore, Maw had no missing data and her genotype was decided based on 7 repeats from 4 samples, 1 hair and 3 faecal. This is may suggest that due to the age of the samples combining results from many repeats (at least 4) different samples, ideally faecal and hair, from the same individual may yield better results with degraded DNA samples.

							95.0 %				
Count	**Degrees of freedom**	**S_VAR**	**S_Std**	**P_Mean**	**S.E**	**t**	**T.Confidence**	**Lower Bound POP VS**	**Upper Bound POP VS**	**95% POP_CI**	**Commit infanticide**
2	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3, 0.3]	commit infanticide
4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide
2	1	1.2%	10.9%	0.0%	7.7%	3.00	98.1%	-74.9%	121.3%	[-0.7,1.2]	inconclusive
1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3, 0.3]	commit infanticide
4	3	10.8%	32.9%	0.0%	16.5%	-0.42	52.4%	-59.4%	45.4%	[-0.6,0.5]	inconclusive
4	3	15.3%	39.2%	0.0%	19.6%	0.07	62.3%	-61.0%	63.7%	[-0.6,0.6]	inconclusive
1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	-100.0%	-100.0%	[-1,-1]	wont commit infanticide
4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide

							95.0%	
Count	Degrees of freedom	S_VAR	S_Std	P_Mean	S.E	t	T.Confidence	Lower Bou POP VS
2	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%
4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%
2	1	1.2%	10.9%	0.0%	7.7%	3.00	98.1%	-74.9%
1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%
4	3	10.8%	32.9%	0.0%	16.5%	- 0.42	52.4%	-59.4%
4	3	15.3%	39.2%	0.0%	19.6%	0.07	62.3%	-61.0%
1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	-100.0%
4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%

Discussion

From the results a few conclusions can be made. First, that there is definitely a relationship between rank and paternity success. This was confirmed by the Rank GLMM where rank was a predictor of paternity success, and with PoA T-Test were ES (partly rank determined) was statistically the same as OS. Added to this, based on a qualitative review of the results, alpha males sired the most offspring (7/12). This difference was most profound in Guenon, where Marcelo sired all the assigned offspring in his group (2/2). However, there were no faecal samples for Chango, the only other male in the group. Similarly the alpha male of Macuco, Ernesto sired the highest proportion of offspring in his group (4/8). In Spot, however, both the alpha (Trucho) and gamma (Tetro) sired one offspring each. Although concrete conclusions cannot be made from this limited dataset, it seems that alphas may be the most successful males. This is corroborated by previous findings in the Iguazú population where females were found to prefer alphas and utilize strategies to bias paternity towards them (Di Bitetti and Janson, 2001a; Ramírez-Llorens *et al.*, 2008; Janson, Baldovino and Bitetti, 2012; Tiddi, Wheeler and Heistermann, 2015a; Tiddi *et al.*, 2018). Furthermore, this trend is seen more widely in the species (*Sapajus nigritus*) (Izar *et al.*, 2009), the genus (*Sapajus apella/macrophelus*- see Methodology: Study Subjects) (Janson, 1984) and the Cebidae family (*Cebus capucinus*) more generally (Muniz *et al.*, 2010). Therefore, the known female alpha preference seems to translate to increased alpha reproductive success.

An unusual finding, however, is the fact that not only did no betas sire, but it was the gammas and deltas who received shares of paternity. Not only this but Sergio, Tetro and Serpico, were all betas at some point during the study period. Anecdotal this hints at the fact that through the females' counterstrategy of paternity confusion, indiscriminate mating may occur with non-alpha males. this may mean that aside among non-alpha males there could be no rank-based differential success. This finding is at odds with the Priority of Access Model, where after the alphas, betas are expected to have the highest share of offspring (Altmann *et al.*, 1996). The reproductive success of these males

may therefore be linked to other factors. For example, male age is often included in statistical analysis of the PoA (Boesch *et al.*, 2006; Newton-Fisher *et al.*, 2010), as it is related to fertility which can affect findings if not accounted for. In this population, however, due to the fact that males disperse multiple times over presumed wide ranges (at least from further than the study site in Iguazú) true male age is not known and it can only be approximated (Janson, Baldovino and Bitetti, 2012, B. Wheeler, personal communication). Following the study period, Sergio briefly became alpha of Macuco (B. Wheeler, personal communication). This may suggest that non-alpha males who gain a share of reproductive success, may be successful as they may possess the beneficial traits which may make them more alluring to the females. Conversely, these traits may be related to the unknown trait which causes alphas to be the most preferred male. Therefore, it may be possible to predict future alphas based on paternity success when lower ranking.

Furthermore, anecdotal evidence suggests that the males who become alphas can come from a range of lower ranks. For example, in Macuco following the disappearance/death of ALS76 (Alessandro: previous alpha, Ernesto was promoted from rank 5 to alpha (Scarry and Tujague, 2012b). Similarly, Alessandro went from being a gamma to an alpha and Marcelo went from being a delta to an alpha (B. Wheeler, personal communication). This is not a surprising observation when it is considered that following alpha takeovers, most of the male group members disperse to find another group (Janson, Baldovino and Bitetti, 2012). This means that any remaining males have a chance to become alphas and solidify their position before any new immigrants arrive.

Due to the limited data set, the PoA model could not be tested, and was therefore neither supported nor refuted. On the one hand, the expected success value was demonstrated to be statistically similar to the observed success, which provides a minimal amount of support for the model. This is because, the expected success value was derived from the female synchrony and male rank predictions of the PoA. On the other hand, the strength of associations varied throughout the years, especially when non-alpha success was greater than alpha success. This suggests that the PoA may not be the best model to run in black horned capuchins. Added to this their sexual behaviour is

characterised by female proceptive behaviour and an apparent absence of mate guarding (Dubuc *et al.*, 2011; Tiddi, Wheeler and Heistermann, 2015a; Bernaldo de Quirós *et al.*, 2018; Tiddi *et al.*, 2018). This means that in *Sapajus nigritus* it is the mainly the females who control sexual behaviour. Through proceptive displays, females approach males to solicit mating. Often when the alpha is being solicited, females will continuously follow him around until he mates with her (Janson, 1984). Likewise it is females to determine the timing of the matings during her peri-ovulatory period. In addition, males often do not constrain female mating, and instead allow females to mate with other males (Janson, 1984, B. Wheeler, personal communication). Female capuchins are thus very much in control of their reproductive success. In contrast to this are catarrhines who subject to more indirect female choice, coercion and mate guarding than platyrrhines (Van Schaik, Hodges and Nunn, 2000; Clarke, Pradhan and Van Schaik, 2009). The PoA was first tested in catarrhine primate, the yellow baboon (*Papio cynocephalus*) (Altmann *et al.*, 1996).This means that despite, the limited dataset, part of the failure of the PoA GLMM could have been the fact that the model predictions differ from true capuchin mating behaviour.

It was determined that throughout the years, females were not confusing paternity enough to prevent infanticide. This is corroborated Ramírez-Llorens *et al.'s* (2008) study where infanticide was observed in the Iguazú population during his 1995 to 2006 study period. As infanticide occurs in the population at a high rate (Ramírez-Llorens *et al.*, 2008) during takeover years, it is fair to say that females are not confusing paternity enough. However, based on my findings, male infanticide tendency varied throughout the years. In addition, based on my limited sample size, males only had to sire one infant in that year to be less likely to commit infanticide. This, however, is a very preliminary finding, as paternity assignment rate was low, so less than half of the infants tested for paternity were included in statistical analysis.

Another reason for the infanticide finding, relates to the relatively long alpha male tenure in black horned capuchins (Janson, Baldovino and Bitetti, 2012). One female counterstrategy in the context of long alpha tenures, is to bias paternity towards alphas to increase the likelihood of receiving

infant protection from infanticide and paternal investment (Clarke, Pradhan and Van Schaik, 2009; Palombit, 2015). This is especially relevant in this study, as alpha rank was stable throughout the years. Therefore, at the time of the study, the current risk for infanticide was greatly reduced and indeed no infanticide occurred (as far as MMS has been told). Furthermore, the female alpha male preference seen in this species, may be due to this counterstrategy. Females may be reducing promiscuity, although not completing stopping this paternity confusion counterstrategy, due to a perhaps more effective alpha protection counterstrategy. Added to this, it was found that in takeover years, female black horned capuchins increased their sexual behaviour, even copulating while pregnant (Ramírez-Llorens *et al.*, 2008). These findings may indicate that females flexibly adopt different strategies over the years. Furthermore, the years in which Sergio, Serpico and Tetro sired offspring could be related to years when females (individual or whole group), mated more promiscuously due to perceived infanticide risk. However, more data is needed to test this prediction.

The eight (Ceb03, Ceb08, Ceb09, Ceb11, Ceb119, Ceb120, Ceb121 and Ceb130) of the microsatellites used in this study (from *Cebus capucinus)* (Muniz and Vigilant, 2008) had previously been evaluated in a Brasilian population of *Sapajus nigritus* (Tokuda, 2012; Tokuda, Martins and Izar, 2014). In this study, of the 18 markers tried, 16 loci successfully amplified, although one of these loci (Ceb128) was monomorphic. In addition, 8 (Ceb02, Ceb03, Ceb08, Ceb10, Ceb105, Ceb11, Ceb121, Ceb127) of 15 loci run through allele checking in Cervus, had high null allele frequencies. In the Brasilian population, Ceb08, Ceb09, Ceb119, Ceb120 and Ceb121, did not amplify (Tokuda, 2012; Tokuda, Martins and Izar, 2014). In this study, Ceb120 also did not amplify. MMS also experienced problems in the form of high null allele frequencies in Ceb08 and Ceb121. Interestingly, in this study, as well as having a high NAF, Ceb121 had the highest number of alleles over a wide range (Methodology: Data Analysis) and also the most mismatches in Cervus (Appendix G: Cervus Outputs). This lead to Ceb121 being the first locus to be excluded in Cervus paternity analysis. Ceb121 also had a high percentage of missing data (Methodology: Data Analysis). Due to all of these problems, the findings of this study

further corroborate the Brasilian one (Tokuda, 2012; Tokuda, Martins and Izar, 2014) that Ceb121 should be excluded from analysis in *Sapajus nigritus*.

However, MMS did not experience any amplification problems with Ceb09 and Ceb119. This may have been due to this study's larger sample size of 70 individuals, consisting of infants and adults, from three groups, versus the 20 individuals, consisting of subadults and adults, from three groups of the Brasilian population (Tokuda, 2012; Tokuda, Martins and Izar, 2014). For the Brasilian population, Ceb03 (N_a=2), Ceb11 (N_a=2) and Ceb130 (N_a=8), amplified. In this study, Ceb03 which only had two alleles in the Brasilian population, and Ceb11 which had two alleles in both populations, had null allele frequencies of more than 0.05. Finally, this study found no problems with Ceb130, which amplified the best in the Brasilian population (Tokuda, 2012; Tokuda, Martins and Izar, 2014). The differences in results between these studies elucidate how sample size affects results. These differences also illustrate how faecal samples can produce variable results even in two populations of the same species.

Using four to seven repeats when genotyping individuals is essential, especially when using poor quality DNA (Taberlet *et al.*, 1996). This is because poor quality DNA produces very variable results as MMS found. What was learnt from this study, which used samples when they were five to twelve years old, is that using a combination of faecal and hair samples yielded the best results. This is because, this combats the problem of only using samples with very degraded DNA, which do not amplify well. Added to this, using at least four repeats to verify both homozygous and heterozygous alleles can greatly reduce genotyping error.

Limitations

Most of the limitations of this study relate to time and budget constraints faced during lab work. Due to the age of the samples, more reliable results would have been obtained had more repeats been performed. This method would have decreased the overall genotyping error rate, and may have lead to more paternity assignments. In addition, during genotyping it was noticed that some plates had negative control contamination and that extractions 14 and 15 did not amplify well, being able to return to the lab to re-do these samples would have also decreased the error rate. More than half of the microsatellites were found to have high NAFs. Therefore, using other markers like those created for *Sapajus nigritus* may have decreased genotyping error (Escobar-Páramo, 2000; Tokuda, Martins and Izar, 2014).

Data analysis was limited by the small numbers of males and years tested. Therefore, during the fieldwork stage it would have been nice to collect samples on more males from more groups over a longer period of time. Furthermore, in the expected success calculation, number of ovulating females was based solely on hormonally verified conceptive cycles. This is a conservative estimate of the true number of ovulating females which were present. Therefore, it may have been useful to calculate this value using all information on all females who conceived during the mating season to use a value that is closer to observed behaviour. For the priority of access model, it would have been useful to include further fertility related factors like age, female parity.

Further Research

It would be interesting to understand results from further paternity tests from black horned capuchin monkeys. This would further findings on male reproductive success. Furthermore, a test of the PoA model on a larger sample size, to determine whether it accurately models capuchin behaviour, would be of interest. Furthermore, future researchers could determine what underlies the alpha male preference and whether it relates to infanticidal counterstrategies at all. Additionally, a further look into whether female promiscuity levels vary in relation to infanticide risk. Factors affecting capuchin fertility in both sexes should also be studied to gain a better understanding on how to model capuchin sexual behaviour. An especially useful target for further research would be how copulation rates and paternity success relates to one another. In addition, exploring the differences in copulation and paternity success among males would also be a fruitful endeavour.

Further research into the transferability of microsatellite markers would also be helpful. It would be especially useful if it could be determined up which taxonomic level, microsatellites stop amplifying. In addition, a study exploring the best way to transfer microsatellite in capuchins would be helpful. Furthermore, another aspect for future research, is how reliable results from a combination of samples can be.

Conclusions

In conclusion, in *Sapajus nigritus*, the female preference for alpha males seems to relate to alpha male paternity success. Although, rank and expected success were both found to relate to paternity success, this relationship was variable with non-alpha males. Females did not confuse paternity enough to prevent infanticide. Looking at individual years, however, when non-alpha males sired offspring, they were not likely to commit infanticide. This means overall females did not confuse paternity enough, but did in some years. During the study period there was no risk of infanticide and thus females may have been mating less promiscuously to bias paternity towards alphas. Therefore, the alpha male preference may be a female counterstrategy. Microsatellites developed for another species, aged faecal samples were used in this study, as well as only two repeats, were used in this study. This means that the high genotyping error and low paternity assignment which affected statistical analysis would have been avoided if more repeats across a combination of samples were performed.

References

Altmann, J. *et al.* (1996) 'Behavior predicts genes structure in a wild primate group.', *Proceedings of the National Academy of Sciences*, 93(12), pp. 5797–5801.

Améndola-Pimenta, M. *et al.* (2009) 'Noninvasive collection of fresh hairs from free-ranging howler monkeys for DNA extraction', *American Journal of Primatology: Official Journal of the American Society of Primatologists*, 71(4), pp. 359–363.

Bateman, A.J. (1948) 'Intra-sexual selection in Drosophila', *Heredity*, 2(3), pp. 349–368.

Bateson, M. and Martin, P. (2021) *Measuring Behaviour: An Introductory Guide*, *Higher Education from Cambridge University Press*. Cambridge University Press. Available at: https://doi.org/10.1017/9781108776462.

Bellemain, E. *et al.* (2006) 'The Dilemma of Female Mate Selection in the Brown Bear, a Species with Sexually Selected Infanticide', *Proceedings: Biological Sciences*, 273(1584), pp. 283–291.

Bellemain, E., Swenson, J.E. and Taberlet, P. (2006) 'Mating Strategies in Relation to Sexually Selected Infanticide in a Non-Social Carnivore: the Brown Bear', *Ethology*, 112(3), pp. 238–246. Available at: https://doi.org/10.1111/j.1439-0310.2006.01152.x.

Bergman, T.J., Ho, L. and Beehner, J.C. (2009) 'Chest color and social status in male geladas (Theropithecus gelada)', *International Journal of Primatology*, 30(6), pp. 791–806.

Bernaldo de Quirós, E. *et al.* (2018) 'Do sexual calls in female black capuchin monkeys (Sapajus nigritus) vary with fertility? An acoustic analysis', *American Journal of Primatology*, 80(9), p. e22920.

Boesch, C. *et al.* (2006) 'Male competition and paternity in wild chimpanzees of the Taï forest', *American Journal of Physical*

Anthropology, 130(1), pp. 103–115. Available at: https://doi.org/10.1002/ajpa.20341.

Boyko, R.H. and Marshall, A.J. (2009) 'The willing cuckold: optimal paternity allocation, infanticide and male reproductive strategies in mammals', *Animal Behaviour*, 77(6), pp. 1397–1407.

Brown, A.D. and Zunino, G.E. (1990) 'Dietary variability in Cebus apella in extreme habitats: evidence for adaptability', *Folia primatologica*, 54(3–4), pp. 187–195.

Campbell, C.J. (2011) *Primates in perspective*. Second edition. Oxford University Press.

Clarke, P., Pradhan, G. and Van Schaik, C.P. (2009) 'Infanticide, Paternity Allocation', *Sexual coercion in primates and humans: an evolutionary perspective on male aggression against females*, p. 42.

Clay, Z. *et al.* (2011) 'Female bonobos use copulation calls as social signals', *Biology Letters*, 7(4), pp. 513–516.

Clay, Z. (2011) *Vocal communication in bonobos (Pan paniscus): studies in the contexts of feeding and sex*. PhD Thesis. University of St Andrews.

Clay, Z. and Zuberbühler, K. (2012) 'Communication during sex among female bonobos: effects of dominance, solicitation and audience', *Scientific Reports*, 2(1), pp. 1–8.

Clutton-Brock, T. (2007) 'Sexual selection in males and females', *Science*, 318(5858), pp. 1882–1885.

Cohas, A. *et al.* (2006) 'Extra-pair paternity in the monogamous alpine marmot (Marmota marmota): the roles of social setting and female mate choice', *Behavioral Ecology and Sociobiology*, 59(5), pp. 597–605.

Constable, J.L. *et al.* (2001) 'Noninvasive paternity assignment in Gombe chimpanzees', *Molecular Ecology*, 10(5), pp. 1279–1300. Available at: https://doi.org/10.1046/j.1365-294X.2001.01262.x.

Cowlishaw, G. and O'Connell, S.M. (1996) 'Male–male competition, paternity certainty and copulation calls in female baboons', *Animal behaviour*, 51(1), pp. 235–238.

Darwin, C. (1859) *On the Origin of Species*. PF Collier & son New York.

Darwin, C. (1871) *The descent of man, and selection in relation to sex*. John Murray.

De Ruiter, J.R., Hooff, J.A.R.A.M.V. and Scheffrahn, W. (1994) 'Social and Genetic Aspects of Paternity in Wild Long-Tailed Macaques (Macaca fascicularis)', *Behaviour*, 129(3/4), pp. 203–224.

De Waal, F. (1997) *Bonobo: The Forgotten Ape*. First Edition. Berkeley: University of California Press.

Di Bitetti, M.S. (1997) 'Evidence for an important social role of allogrooming in a platyrrhine primate', *Animal behaviour*, 54(1), pp. 199–211.

Di Bitetti, M.S. and Janson, C.H. (2001a) 'Reproductive socioecology of tufted capuchins (Cebus apella nigritus) in northeastern Argentina', *International Journal of Primatology*, 22(2), pp. 127–142.

Di Bitetti, M.S. and Janson, C.H. (2001b) 'Social foraging and the finder's share in capuchin monkeys, Cebus apella', *Animal Behaviour*, 62(1), pp. 47–56. Available at: https://doi.org/10.1006/anbe.2000.1730.

Di Fiore, A. *et al.* (2009) 'Dispersal patterns in sympatric woolly and spider monkeys: integrating molecular and observational data', *Behaviour*, 146(4–5), pp. 437–470.

Dixson, A.F. (2012) *Primate sexuality comparative studies of the prosimians, monkeys, apes and humans*. 2nd ed.

Dubuc, C. *et al.* (2011) 'Testing the priority-of-access model in a seasonally breeding primate species', *Behavioral Ecology and Sociobiology*, 65(8), pp. 1615–1627. Available at: https://doi.org/10.1007/s00265-011-1172-8.

Escobar-Páramo, P. (1999) *Inbreeding avoidance and the evolution of male mating strategies*. State University of New York at Stony Brook.

Escobar-Páramo, P. (2000) 'Microsatellite primers for the wild brown capuchin monkey Cebus apella', *Molecular Ecology*, 9(1), pp. 107–108.

Feh, C. and Munkhtuya, B. (2008) 'Male infanticide and paternity analyses in a socially natural herd of Przewalski's horses: sexual selection?', *Behavioural Processes*, 78(3), pp. 335–339.

Fleagle, J.G. (1988) *Primate adaptation & evolution*.

Fossey, D. (2000) *Gorillas in the Mist*. Houghton Mifflin Harcourt.

Galdikas, B.M. (1995) 'Reflections of Eden: My life with the orangutans of Borneo'.

Gerald, M.S. (2001) 'Primate colour predicts social status and aggressive outcome', *Animal Behaviour*, 61(3), pp. 559–566. Available at: https://doi.org/10.1006/anbe.2000.1648.

Gerloff, U. *et al.* (1999) 'Intracommunity Relationships, Dispersal Pattern and Paternity Success in a Wild Living Community of Bonobos (Pan paniscus) Determined from DNA Analysis of Faecal Samples', *Proceedings: Biological Sciences*, 266(1424), pp. 1189–1195.

Glenn, T.C. and Schable, N.A. (2005) 'Isolating microsatellite DNA loci', in *Methods in enzymology*. Elsevier, pp. 202–222.

Goossens, B. *et al.* (1998) 'Extra-pair paternity in the monogamous Alpine marmot revealed by nuclear DNA microsatellite analysis', *Behavioral Ecology and Sociobiology*, 43(4), pp. 281–288.

Gowaty, P.A. and Hubbell, S.P. (2005) 'Chance, time allocation, and the evolution of adaptively flexible sex role behavior', *Integrative and Comparative Biology*, 45(5), pp. 931–944.

Hamilton III, W.J. and Arrowood, P.C. (1978) 'Copulatory vocalizations of chacma baboons (Papio ursinus), gibbons (Hylobates hoolock), and humans', *Science*, 200(4348), pp. 1405–1409.

Henzi, S.P. (1996) 'Copulaton calls and paternity in chacma baboons', *Animal Behaviour*, 51, pp. 233–234.

Hrdy, S.B. (1974) 'Male-male competition and infanticide among the langurs (Presbytis entellus) of Abu, Rajasthan', *Folia primatologica*, 22(1), pp. 19–58.

Hrdy, S.B. and Hausfater, G. (1984) 'Comparative and evolutionary perspectives on infanticide: introduction and overview', *Infanticide: comparative and evolutionary perspectives*, pp. xiii–xxxv.

IBM Corp (2021) 'IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp'.

Izar, P. *et al.* (2009) 'Sexual selection, female choice and mating systems', in *South American Primates*. Springer, pp. 157–189.

Janson, C. (1985) 'Aggresive competition and individual food consumption in wild brown capuchin monkeys (Cebus apella)', *Behavioral Ecology and Sociobiology*, 18(2), pp. 125–138.

Janson, C., Baldovino, M.C. and Bitetti, M.D. (2012) 'The group life cycle and demography of brown capuchin monkeys (Cebus [apella] nigritus) in Iguazú National Park, Argentina', in *Long-term field studies of primates*. Springer, pp. 185–212.

Janson, C.H. (1984) 'Female choice and mating system of the brown capuchin monkey Cebus apella (Primates: Cebidae)', *Zeitschrift für Tierpsychologie*, 65(3), pp. 177–200.

Janson, C.H. (1996) 'Toward an experimental socioecology of primates', in *Adaptive radiations of neotropical primates*. Springer, pp. 309–325.

Janson, C.H. and Van Schaik, C.P. (2000) 'The behavioral ecology of infanticide by males', *Infanticide by males and its implications* [Preprint].

Jones, A.G. *et al.* (2010) 'A practical guide to methods of parentage analysis', *Molecular ecology resources*, 10(1), pp. 6–30.

Kalinowski, S.T., Taper, M.L. and Marshall, T.C. (2007) 'Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment', *Molecular ecology*, 16(5), pp. 1099–1106.

Kalinowski, S.T., Taper, M.L. and Marshall, T.C. (2010) 'Corrigendum', *Molecular Ecology*, 19(7), pp. 1512–1512. Available at: https://doi.org/10.1111/j.1365-294X.2010.04544.x.

Kappeler, P.M. and van Schaik, C.P. (2002) 'Evolution of primate social systems', *International journal of primatology*, 23, pp. 707–740.

Kim, K.S. and Sappington, T.W. (2013) 'Microsatellite data analysis for population genetics', in *Microsatellites*. Springer, pp. 271–295.

Knott, C.D. and Kahlenberg, S. (2007) 'Orangutans in perspective: forced copulations and female mating resistance', in *Primates in perspective*. Oxford University Press.

Lee, A. and Huang, M. (2021) 'Oropendola nest predation and rodent consumption by the black-capped capuchin (Sapajus apella) in the Manu Biosphere Reserve, Peru'. Available at: https://ecoevorxiv.org/repository/view/4113/ (Accessed: 22 November 2022).

Liebal, K. *et al.* (2014) *Primate communication: a multimodal approach*. Cambridge University Press.

Lima, M.G.M. *et al.* (2017) 'Capuchin monkey biogeography: understanding Sapajus Pleistocene range expansion and the current sympatry between Cebus and Sapajus', *Journal of Biogeography*, 44(4), pp. 810–820. Available at: https://doi.org/10.1111/jbi.12945.

Lin, H.-S. and Chang, S.-B. (2013) 'PCR-based isolation of microsatellite arrays (PIMA)', in *Microsatellites*. Springer, pp. 25–55.

Lukas, D. and Huchard, E. (2014) 'The evolution of infanticide by males in mammalian societies', *Science*, 346(6211), pp. 841–844.

Lynch Alfaro, J.W. *et al.* (2012) 'Explosive Pleistocene range expansion leads to widespread Amazonian sympatry between robust and gracile capuchin monkeys', *Journal of Biogeography*, 39(2), pp. 272–288. Available at: https://doi.org/10.1111/j.1365-2699.2011.02609.x.

Lynch Alfaro, J.W., Silva Jr, J.D.S.E. and Rylands, A.B. (2012) 'How different are robust and gracile capuchin monkeys? An argument for the use of Sapajus and Cebus', *American Journal of Primatology*, 74(4), pp. 273–286.

Madesis, P., Ganopoulos, I. and Tsaftaris, A. (2013) 'Microsatellites: evolution and contribution', in *Microsatellites*. Springer, pp. 1–13.

Maestripieri, D. and Roney, J.R. (2005) 'Primate copulation calls and postcopulatory female choice', *Behavioral Ecology*, 16(1), pp. 106–113.

Manu, Manu National Park | Parque Nacional del Manu (no date). Available at: https://www.manuadventures.com/about_the__manu_national_park.html (Accessed: 22 November 2022).

Marshall, T.C. *et al.* (1998) 'Statistical confidence for likelihood-based paternity inference in natural populations', *Molecular ecology*, 7(5), pp. 639–655.

Mayr, E. (ed.) (1972) 'Sexual Selection and Natural Selection', in *Sexual Selection and the Descent of Man*. Routledge.

Møller, A.P. and Birkhead, T.R. (1993) 'Certainty of paternity covaries with paternal care in birds', *Behavioral Ecology and Sociobiology*, 33(4), pp. 261–268. Available at: https://doi.org/10.1007/BF02027123.

Morin, P.A. *et al.* (2001) 'Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (Pan troglodytes verus)', *Molecular Ecology*, 10(7), pp. 1835–1844. Available at: https://doi.org/10.1046/j.0962-1083.2001.01308.x.

Muller, M.N., Kahlenberg, S.M. and Wrangham, R.W. (2009) 'Male aggression and sexual coercion of females in primates', *Sexual coercion in primates and humans: An evolutionary perspective on male aggression against females*, pp. 3–22.

Muniz, L. *et al.* (2010) 'Male dominance and reproductive success in wild white-faced capuchins (Cebus capucinus) at Lomas Barbudal, Costa Rica', *American Journal of Primatology*, 72(12), pp. 1118–1130.

Muniz, L. and Vigilant, L. (2008) 'Isolation and characterization of microsatellite markers in the white-faced capuchin monkey (Cebus capucinus) and cross-species amplification in other New World monkeys', *Molecular Ecology Resources*, 8(2), pp. 402–405.

National Human Genome Research Institute (NHGRI) (2023) *Primer*, *Genome.gov*. Available at: https://www.genome.gov/genetics-glossary/Primer (Accessed: 10 January 2023).

Newton-Fisher, N.E. *et al.* (2010) 'Paternity and social rank in wild chimpanzees (Pan troglodytes) from the Budongo Forest, Uganda', *American Journal of Physical Anthropology*, 142(3), pp. 417–428. Available at: https://doi.org/10.1002/ajpa.21241.

Nikitopoulos, E. *et al.* (2004) 'Influence of female copulation calls on male sexual behavior in captive Macaca fascicularis', *International Journal of Primatology*, 25(3), pp. 659–677.

Nunn, C.L. (1999) 'The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis', *Animal Behaviour*, 58(2), pp. 229–246. Available at: https://doi.org/10.1006/anbe.1999.1159.

O'Connell, S.M. and Cowlishaw, G. (1994) 'Infanticide avoidance, sperm competition and mate choice: the function of copulation calls in female baboons', *Animal behaviour*, 48(3), pp. 687–694.

Palombit, R.A. (2015) 'Infanticide as Sexual Conflict: Coevolution of Male Strategies and Female Counterstrategies', *Cold Spring Harbor Perspectives in Biology*, 7(6), p. a017640. Available at: https://doi.org/10.1101/cshperspect.a017640.

Pereira, M.E. and Weiss, M.L. (1991) 'Female Mate Choice, Male Migration, and the Threat of Infanticide in Ringtailed Lemurs', *Behavioral Ecology and Sociobiology*, 28(2), pp. 141–152.

Perez-Sweeney, B.M. *et al.* (2005) 'Dinucleotide microsatellite primers designed for a critically endangered primate, the black lion tamarin (Leontopithecus chrysopygus)', *Molecular Ecology Notes*, 5(2), pp. 198–201. Available at: https://doi.org/10.1111/j.1471-8286.2005.00875.x.

Pope, T.R. (1990) *The Reproductive Consequences of Male Cooperation in the Red Howler Monkey: Paternity Exclusion in Multi-Male and Single-Male Troops Using Genetic Markers*. Available at: https://www-jstor-org.chain.kent.ac.uk/stable/4600503#metadata_info_tab_contents (Accessed: 21 November 2022).

Pradhan, G.R. *et al.* (2006) 'The evolution of female copulation calls in primates: a review and a new model', *Behavioral Ecology and Sociobiology*, 59(3), pp. 333–343.

Pusey, A.E. and Packer, C. (1994) 'Infanticide in Lions: Consequences and Counterstrategies', in *Infanticide And Parental Care*. Routledge.

Ramírez-Llorens, P. *et al.* (2008) 'Infanticide in black capuchin monkeys (Cebus apella nigritus) in Iguazú National Park, Argentina', *American Journal of Primatology: Official Journal of the American Society of Primatologists*, 70(5), pp. 473–484.

Redmond, I. (2010) *Primates of the world : the amazing diversity of our closest relatives*. New Holland.

Reichard, U.H. (2009) 'The social organization and mating system of Khao Yai white-handed gibbons: 1992-2006', in *The gibbons*. Springer, pp. 347–384.

Ruiz-García, M. *et al.* (2012) 'Molecular Relationships and Classification of Several Tufted Capuchin Lineages (Cebus apella, Cebus xanthosternos and Cebus nigritus, Cebidae), by Means of Mitochondrial Cytochrome Oxidase II Gene Sequences', *Folia Primatologica*, 83(2), pp. 100–125. Available at: https://doi.org/10.1159/000342832.

Ruiz-García, M., Castillo, M. and Luengas-Villamil, K. (2016) 'It is misleading to use Sapajus (robust capuchins) as a genus? : A review of the evolution of the capuchins and suggestions on their systematics', in.

Scarry, C.J. (2013) 'Between-group contest competition among tufted capuchin monkeys, Sapajus nigritus, and the role of male resource defence', *Animal Behaviour*, 85(5), pp. 931–939. Available at: https://doi.org/10.1016/j.anbehav.2013.02.013.

Scarry, C.J. and Tujague, M.P. (2012a) 'Consequences of Lethal Intragroup Aggression and Alpha Male Replacement on Intergroup Relations and Home Range Use in Tufted Capuchin Monkeys (Cebus apella nigritus)', *American Journal of Primatology*, 74(9), pp. 804–810. Available at: https://doi.org/10.1002/ajp.22030.

Scarry, C.J. and Tujague, M.P. (2012b) 'Consequences of Lethal Intragroup Aggression and Alpha Male Replacement on Intergroup Relations and Home Range Use in Tufted Capuchin Monkeys (Cebus apella nigritus)', *American Journal of Primatology*, 74(9), pp. 804–810. Available at: https://doi.org/10.1002/ajp.22030.

van Schaik, C.P. and Janson, C.H. (eds) (2000) *Infanticide by Males and its Implications*. Cambridge: Cambridge University Press. Available at: https://doi.org/10.1017/CBO9780511542312.

Seyfarth, R.M. (1980) 'The distribution of grooming and related behaviours among adult female vervet monkeys', *Animal Behaviour*, 28(3), pp. 798–813. Available at: https://doi.org/10.1016/S0003-3472(80)80140-0.

Slate, J., Marshall, T. and Pemberton, J. (2000) 'A retrospective assessment of the accuracy of the paternity inference program CERVUS', *Molecular Ecology*, 9(6), pp. 801–808.

Strier, K.B. (2016) *Primate behavioral ecology*. Routledge.

Taberlet, P. *et al.* (1996) 'Reliable genotyping of samples with very low DNA quantities using PCR', *Nucleic acids research*, 24, pp. 3189–94. Available at: https://doi.org/10.1093/nar/24.16.3189.

Thomas, L. and Bell, J.J. (2012) 'Characterization of polymorphic microsatellite markers for the red rock lobster, Jasus edwardsii (Hutton 1875)', *Conservation Genetics Resources*, 4(2), pp. 319–321.

Tiddi, B., Aureli, F., Polizzi di Sorrentino, E., *et al.* (2011) 'Grooming for tolerance? Two mechanisms of exchange in wild tufted capuchin monkeys', *Behavioral Ecology*, 22(3), pp. 663–669.

Tiddi, B., Aureli, F., Schino, G., *et al.* (2011) 'Social relationships between adult females and the alpha male in wild tufted capuchin monkeys', *American Journal of Primatology*, 73(8), pp. 812–820.

Tiddi, B. *et al.* (2018) 'Male resource defense mating system in primates? An experimental test in wild capuchin monkeys', *Plos one*, 13(5), p. e0197020.

Tiddi, B., Aureli, F. and Schino, G. (2010) 'Grooming for infant handling in tufted capuchin monkeys: a reappraisal of the primate infant market', *Animal Behaviour*, 79(5), pp. 1115–1123.

Tiddi, B., Aureli, F. and Schino, G. (2012) 'Grooming up the hierarchy: the exchange of grooming and rank-related benefits in a new world primate', *PloS one*, 7(5), p. e36641.

Tiddi, B., Wheeler, B.C. and Heistermann, M. (2015a) 'Female behavioral proceptivity functions as a probabilistic signal of fertility, not female quality, in a New World primate', *Hormones and Behavior*, 73, pp. 148–155.

Tiddi, B., Wheeler, B.C. and Heistermann, M. (2015b) 'Mating patterns in relation to the timing of ovulation in Argentine black capuchins', *Folia Primatologica*, 86, pp. 371–372.

Tokuda, M. (2012) *Dispersão e estrutura social de macacos-prego (Sapajus nigritus) do Parque Estadual Carlos Botelho, São Paulo*. PhD Thesis. Universidade de São Paulo.

Tokuda, M., Martins, M.M. and Izar, P. (2014) 'Transferability of microsatellites for studies on the social behavior of the tufted capuchin monkey (genus Sapajus)', *Gen Mol Res*, 13, pp. 9910–9914.

Townsend, S.W., Deschner, T. and Zuberbühler, K. (2008) 'Female chimpanzees use copulation calls flexibly to prevent social competition', *PLoS One*, 3(6), p. e2431.

Trivers, R.L. (1972) 'Parental investment and sexual selection', in *Sexual selection and the descent of man*. Routledge, pp. 136–179.

Van Belle, S. *et al.* (2014) 'Social and genetic factors mediating male participation in collective group defence in black howler monkeys', *Animal Behaviour*, 98, pp. 7–17.

Van Belle, S., Estrada, A. and Di Fiore, A. (2014) 'Kin-biased spatial associations and social interactions in male and female black howler monkeys (Alouatta pigra)', *Behaviour*, 151(14), pp. 2029–2057.

Van Schaik, C.P. (2000) 'Infanticide by male primates: the sexual selection hypothesis revisited', *Infanticide by males and its implications* [Preprint].

Van Schaik, C.P., Hodges, J.K. and Nunn, C.L. (2000) 'Paternity confusion and the ovarian cycles of female primates', *Infanticide by males and its implications*, pp. 361–387.

Walling, C.A. *et al.* (2010) 'Comparing parentage inference software: reanalysis of a red deer pedigree', *Molecular Ecology*, 19(9), pp. 1914–1928.

Wheeler, B.C. (2009) 'Monkeys crying wolf? Tufted capuchin monkeys use anti-predator calls to usurp resources from conspecifics', *Proceedings of the Royal Society B: Biological Sciences*, 276(1669), pp. 3013–3018. Available at: https://doi.org/10.1098/rspb.2009.0544.

Wheeler, B.C. (2010) 'Decrease in Alarm Call Response Among Tufted Capuchins in Competitive Feeding Contexts: Possible Evidence for Counterdeception', *International Journal of Primatology*, 31(4), pp. 665–675. Available at: https://doi.org/10.1007/s10764-010-9419-1.

Wheeler, B.C., Tiddi, B. and Heistermann, M. (2014) 'Competition-induced stress does not explain deceptive alarm calling in tufted capuchin monkeys', *Animal Behaviour*, 93, pp. 49–58.

Wolff, J.O. and Macdonald, D.W. (2004) 'Promiscuous females protect their offspring', *Trends in Ecology & Evolution*, 19(3), pp. 127–134.

Zhao, Q., Borries, C. and Pan, W. (2011) 'Male takeover, infanticide, and female countertactics in white-headed leaf monkeys (Trachypithecus leucocephalus)', *Behavioral Ecology and Sociobiology*, 65(8), pp. 1535–1547.

Zinner, D.P. *et al.* (2004) 'Sexual selection and exaggerated sexual swellings of female primates', *Sexual selection in primates: New and comparative perspectives*, pp. 71–89.

Appendices

Appendix A. Study Subjects

Table 35. Showing the study subjects including Individual ID, Sex, Age Class, Mother ID, Birth Date, Group

INDIVIDUAL ID	ID CODE AND NUMBER	SEX	AGE CLASS	MOTHER ID	BIRTH DATE	GROUP
CARMELA	CAR81	F	ADULT			RIT
LILIANA	LIL14	F	ADULT	RITA	LATE 2004	RIT
ELENA	ELE8	F	ADULT	GUENON		RIT
GUENON	GUE49	F	ADULT			RIT
MAYA	MAY18	F	ADULT	GUENON		RIT
RITA	RIT67	F	ADULT	GUENON		RIT
BARDO	BAR38	M	ADULT			RIT
MARCELO	MAR16	M	ADULT			RIT
MERLOT	MER60	M	ADULT			RIT
OCTAVIO	OCT20	M	ADULT			RIT
VELMIRO	VEL33	M	ADULT			RIT
CHANGO	CHA77	M	ADULT			RIT
ALELA	ALE36	F	INFANT	ELENA	15/11/2009	RIT
ILARIA	ILA53	F	INFANT	RITA	15/11/2009	RIT
MILTON	MIL62	M	INFANT	MAYA	2010	RIT
DAVID	DAV44	M	INFANT	RITA	23/12/2006	RIT
HORACIO	HOR51	M	INFANT	ELENA	25/11/2007	RIT
JACK	JAC55	M	INFANT	RITA	25/09/2008	RIT
ELOY	ELO45	M	INFANT	ELENA	15/11/2010	RIT
ISIDRO	ISI54	M	INFANT	LILIANA	15/11/2010	RIT
EDUARDO	EDU7	M	INFANT	CARMELA	LATE 2006	RIT
SEAN	SEA69	M	INFANT	MAYA	LATE 2008	RIT
SERGIO	SER27	M	INFANT			RIT
CHICCA	CHI82	F	ADULT			MAC
MAW	MAW17	F	ADULT	URSULA	LATE 2005	MAC
ESTELA	EST10	F	ADULT	THELMA		MAC
OFELIA	OFE21	F	ADULT	OLIVIA		MAC
SOL	SOL28	F	ADULT			MAC
THELMA	THE30	F	ADULT			MAC
YOLI	YOL35	F	ADULT			MAC
EDUARDO	EDU7	M	ADULT	CARMELA		MAC
ERNESTO	ERN9	M	ADULT			MAC
SERGIO	SER27	M	ADULT			MAC
SERPICO	SRP29	M	ADULT			MAC
TITO	TTO32	M	ADULT			MAC
ALESSANDRO	ALS76	M	ADULT			MAC
HOMERO	HOM78	M	ADULT			MAC
JESUS	JES79	M	ADULT			MAC

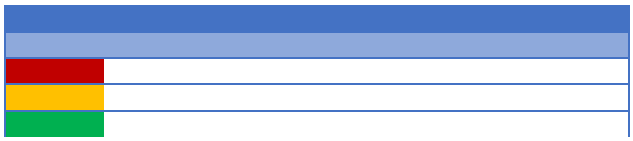
JUSTO	JUS80	M	ADULT			MAC
GUIDITTA	GUI50	F	INFANT	CHICCA	08/12/2006	MAC
OMAR	OMA22	M	INFANT	INGRID	2010	MAC
LENIN	LEN57	M	INFANT	THELMA	2011	MAC
ZISSOU	ZIS75	M	INFANT	ESTELA	2011	MAC
PIETRO	PIE25	M	INFANT	YOLI	2012	MAC
JUAN CARLOS	JC56	M	INFANT	CORDELIA	2013	MAC
SIMON	SIM70	M	INFANT	INGRID	2013	MAC
MAGOO	MGO61	M	INFANT	CLARA	09/11/2006	MAC
DALI	DAL42	M	INFANT	YOLI	04/11/2007	MAC
MAVERICK	MAV59	M	INFANT	ESTELA	17/10/2008	MAC
FRANCISCO	FRA48	M	INFANT	SPOT	01/12/2008	MAC
RICKY	RIC66	M	INFANT	THELMA	25/12/2008	MAC
CAMILO	CAM41	M	INFANT	JOSEFA	02/11/2009	MAC
FENY	FEN47	M	INFANT	YOLI	15/11/2009	MAC
ASTOR	AST1	M	INFANT	YOLI	24/11/2010	MAC
ESTEBAN	ESB46	M	INFANT	SOL	08/02/2011	MAC
CAMBAI	CAB40	M	INFANT	THELMA	15/12/2013	MAC
ROBERTO	ROB68	M	INFANT	CLARA	LATE 2009	MAC
CORDELIA	COR5	F	JUV/ADULT	CHI	LATE 2008	MAC
CLARA	CLA4	F	ADULT	SPOT		MAC/SPO
EVA	EVA11	F	ADULT	CLARA		MAC/SPO
JOSEFA	JOS13	F	ADULT	SPOT		MAC/SPO
TRUCHO	TRU31	M	ADULT			MAC/SPO
BIANCA	BIA2	F	ADULT	SPOT	LATE 2003	SPO
DANIELA	DAN6	F	ADULT	SPOT	LATE 2005	SPO
INGRID	ING12	F	ADULT	YOLI	LATE 2005	SPO
SPOT	SPO72	F	ADULT			SPO
PACINO	PAC24	M	ADULT			SPO
RINGO	RIN26	M	ADULT			SPO
TETRO	TET74	M	ADULT			SPO
SPARTACUS	SPC71	M	INFANT	SPOT	2010	SPO
ANIBAL	ANI37	M	INFANT	DANIELA	2012	SPO
HURLIO	HUR52	M	INFANT	EVA	2012	SPO
MATILDA	MAT58	M	INFANT	MAW	2012	SPO
MOJO	MOJ63	M	INFANT	BIANCA	2012	SPO
MURTANG	MUR65	M	INFANT	SOL	2012	SPO
BEAU	BEA39	M	INFANT	BIANCA	15/11/2010	SPO
DANTE	DAT43	M	INFANT	DANIELA	01/01/2011	SPO
SYRAH	SYR73	M	INFANT	JOSEFA	01/11/2011	SPO
BORAT	BOR3	M	ADULT			SPO/MAC?

Note:
Individuals in purple: no DNA was extracted from these adult females who conceived offspring
Individuals in blue: no DNA was extracted from these adult males who are potential fathers

Appendix B. PCR and Gel Tracker

Table 36. Showing a sample of the PCR and gel tracker used from January 2022

Date of PCR	PCR #	Markers amplified	PCR Comments	Date of gel	Gel Name	traction #	Gel Status	Gel Comments
24/01/2022	51	Ceb 130	ext 5 (n=15)	& 28/01/2022	PCR51a an	5		OCT (1 sample) (PCR51a)- OCT & SER (2 samples) (PCR51b) amplified
27/01/2022	52	Ceb 120	ext 5 (n=15)	28/01/2022	PCR52a	5		OCT, AST, MARC & SER (4 samples) amplified
27/01/2022	52	Ceb 128	ext 5 (n=15)	28/01/2022	PCR52b	5		MARC & SER (2 samples) amplified
28/01/2022	53	Ceb 11	ext 5 (n=15)	07/02/2022	PCR53c	5		OCT (1 sample) amplified
28/01/2022	53	Ceb 121	ext 5 (n=15)	07/02/2022	PCR53b	5		
28/01/2022	53	Ceb 130	ext 5 (n=15)	07/02/2022	PCR53a	5		
07/02/2022	54	Ceb 120	ext 5 (n=15), PCR with 4ul DNA	08/02/2022	PCR54a	5		SER (1 sample) amplified
07/02/2022	54	Ceb 128	ext 5 (n=15), PCR with 4ul DNA	08/02/2022	PCR54a	5		
16/02/2022	55	Ceb 02	ext 6 (n=5)	17/02/2022	PCR55	6		
16/02/2022	55	Ceb 07	ext 6 (n=5)	17/02/2022	PCR55	6		maybe AST (1 sample) amplified
16/02/2022	56	Ceb 11	ext 6 (n=5)	17/02/2022	PCR56	6		
16/02/2022	56	Ceb 121	ext 6 (n=5)	17/02/2022	PCR56	6		
16/02/2022	56	Ceb 130	ext 6 (n=5)	17/02/2022	PCR56	6		
17/02/2022	57	Ceb 08	ext 6 (n=5)	25/02/2022	PCR 57	6		maybe AST (1 sample) amplified
17/02/2022	57	Ceb 115	ext 6 (n=5)	25/02/2022	PCR 57	6		
17/02/2022	57	Ceb 03	ext 6 (n=5)	25/02/2022	PCR 57	6		maybe AST (1 sample) amplified
17/02/2022	58	Ceb 09	ext 6 (n=5)	25/02/2022	PCR 58	6		
17/02/2022	58	Ceb 105	ext 6 (n=5)	25/02/2022	PCR 58	6		OCT & MARC (2 samples) amplified
17/02/2022	58	Ceb 127	ext 6 (n=5)	25/02/2022	PCR 58	6		
17/02/2022	58	Ceb 128	ext 6 (n=5)	25/02/2022	PCR 58	6		OCT & MAY (2 samples) amplified
25/02/2022	59	Ceb 02	ext 1 (n=4), ext 2 (n=3), ext 3 (n=4), ext 4-hair (n=3)	25/02/2022	PCR 59a	1, 2, 3, 4		
25/02/2022	59	Ceb 07	ext 1 (n=4), ext 2 (n=3), ext 3 (n=4), ext 4-hair (n=3)	25/02/2022	PCR 59a &	1, 2, 3, 4		
28/02/2022	60	Ceb 02	ext 1 (n=5), ext 2 (n=4), ext 3 (n=5)	02/03/2022	PCR60a	1, 2, 3		
28/02/2022	60	Ceb 07	ext 1 (n=5), ext 2 (n=4), ext 3 (n=5)	02/03/2022	PCR60b	1, 2, 3		
28/02/2022	61	Ceb 11	ext 1 (n=9), ext 2 (n=6), ext 3 (n=5)	02/03/2022	PCR61a	1, 2, 3		
28/02/2022	61	Ceb 121	ext 1 (n=9), ext 2 (n=6), ext 3 (n=5)	03/03/2022	PCR61b	1, 2, 3		
28/02/2022	61	Ceb 130	ext 1 (n=9), ext 2 (n=3), ext 3 (n=4)	03/03/2022	PCR61c	1, 2, 3		
02/03/2022	62	Ceb 04	ext 1 (n=9), ext 2 (n=2), ext 3 (n=3)	03/03/2022	PCR62	1, 2, 3		
02/03/2022	63	Ceb 10	ext 1 (n=9), ext 3 (n=2)- PCR was prepared and then put in fridge for	04/03/2022	PCR63	1, 3		
02/03/2022	64	Ceb 105	ext 1 (n=9)	04/03/2022	PCR64b	1		



Appendix C. Durham University Plate Design

Table 37. Showing general full plate design and how wells with Post-PCR multiplexes were denoted using Plate 53 as an example

Plate 53	Ceb128	Ceb03	Ceb08	Ceb115	Ceb130	Ceb127	Ceb07	Ceb09	Ceb105
	N = 11	N = 19	N = 8	N = 3		N = 8	N = 1NC		
	PCR 109	PCR 110			PCR 116	PCR 113	PCR 112	PCR 113	PCR 115
	On exts 10, 11	On exts 12, 13							

	1		2		3		4		5		6		7		8		9		10		11		12	
A	Tet	10	Tet	10	Mil	11	Mil	11	Esb	12	Esb	12	Spc	13	Spc	13	Bea	12	Bea	12	Len	13	Len	13
	128	50	128	100	128	50	128	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	08 127	50	08 127	100
B	Gue	11	Gue	11	Sea	11	Sea	11	Fen	12	Fen	12	Zis	13	Zis	13	Cam	12	Cam	12	Sim	13	Sim	13
	128	50	128	100	128	50	128	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	08 127	50	08 127	100
C	Ila	11	Ila	11	Spo	11	Spo	11	Fra	12	Fra	12	Hur	13	Hur	13	Hor	12	Hor	12	Ani	13	Ani	13
	128	50	128	100	128	50	128	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	08 127	50	08 127	100
D	Gui	11	Gui	11	NC-PCR110		NC-PCR110		Mav	12	Mav	12	Mo r	13	Mo r	13	Moj	12	Moj	12	NC-PCR116		NC-PCR112	
	128	50	128	100	03	50	08	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	130	50	07	100
E	Isi	11	Isi	11	NC-PCR110		NC-PCR113		Bea	12	Bea	12	Dat	13	Dat	13	Dat	13	Dat	13	NC-PCR113		NC-PCR115	
	128	50	128	100	115	50	127	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	09	50	105	100
F	Jac	11	Jac	11	Len	13	Len	13	Cam	12	Cam	12	JC	13	JC	13	JC	13	JC	13	Len	13	Len	13
	128	50	128	100	03	50	03	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	130 115	50	130 115	100
G	Mer	11	Mer	11	Sim	13	Sim	13	Hor	12	Hor	12	Ric	13	Ric	13	Ric	13	Ric	13	Sim	13	Sim	13
	128	50	128	100	03	50	03	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	130 115	50	130 115	100
H	Rit	11	Rit	11	Ani	13	Ani	13	Moj	12	Moj	12	Syr	13	Syr	13	Syr	13	Syr	13	Ani	13	Ani	13
	128	50	128	100	03	50	03	100	03	50	03	100	03	50	03	100	08 127	50	08 127	100	130 115	50	130 115	100

Note:

For each well in the plate design, the top left corner was written in dark blue ink denoting Individual ID, the top right was red denoting extraction (ext) number, the bottom left was green denoting the microsatellite number and the bottom right was coloured black denoting the dilution (50 = 1:50, 100 = 1:100).

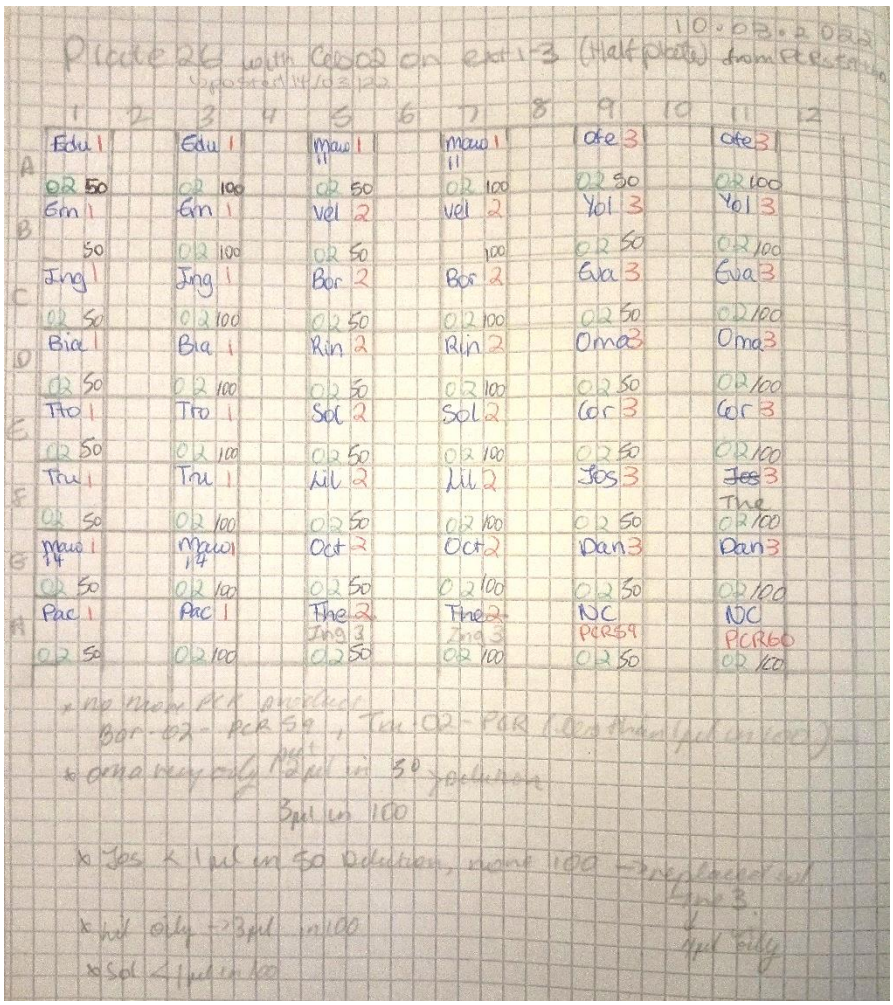


Figure 5. Showing half Plate 26 as an example of half plates and during lab notes

Appendix D. Post-Geneious Excel Outputs

Table 38. Showing an example (Ceb08-Plates 39 & 53) of MMS' post Geneious excel file

ID names	DNA Extraction	Plate	Dilutions	Plate Comments	Ceb08- 1	Ceb08- 2	Geneious Name	Geneious Comments
ANI	13	53	50		177	177	C11_300622d-Plate53	
ANI	13	53	100		177	177	C12_300622d-Plate53	
BEA	12	53	50		177	177	A09_300622d-Plate53	Peak 177 under 600 RFU
BEA	12	53	100		177	177	A10_300622d-Plate53	
CAM	12	53	50		173	181	B09_300622d-Plate53	
CAM	12	53	100		173	181	B10_300622d-Plate53	
DAT	13	53	50		189	189	E09_300622d-Plate53	Peak 189 under 700 RFU
DAT	13	53	100		189	189	E10_300622d-Plate53	
ESB	12	39	20		169	177	A01_230622c-Plate39	
ESB	12	39	50		169	177	A02_230622c-Plate39	
ESB	12	39	100	<1 µl added	177	177	A03_230622c-Plate39	Peak 177 ~500 RFU
FEN	*12*	*39*	*20*		*157*	*185*	*B01_230622c-Plate39*	
FEN	*12*	*39*	*50*		*157*	*185*	*B02_230622c-Plate39*	
FEN	*12*	*39*	*100*	<1 µl added	*No peaks*	*No peaks*	*B03_230622c-Plate39*	
FRA	12	39	20		177	177	C01_230622c-Plate39	
FRA	12	39	50		177	177	C02_230622c-Plate39	
FRA	12	39	100	<1 µl added	177	177	C03_230622c-Plate39	Peak 177 ~500 RFU
HOR	12	53	50		169	181	C09_300622d-Plate53	
HOR	12	53	100		169	181	C10_300622d-Plate53	
HUR	13	39	20		169	177	G01_230622c-Plate39	
HUR	13	39	50		169	177	G02_230622c-Plate39	
HUR	13	39	100	<1 µl added	169	177	G03_230622c-Plate39	Peaks under 700 RFU
JC	13	53	50		No peaks	No peaks	F09_300622d-Plate53	
JC	13	53	100		No peaks	No peaks	F10_300622d-Plate53	
LEN	13	53	50		No peaks	No peaks	A11_300622d-Plate53	
LEN	13	53	100		No peaks	No peaks	A12_300622d-Plate53	
MAV	12	39	20		157	169	D01_230622c-Plate39	
MAV	12	39	50		157	169	D02_230622c-Plate39	
MAV	12	39	100		157	169	D03_230622c-Plate39	
MOJ	12	53	50		161	177	D09_300622d-Plate53	
MOJ	12	53	100		161	177	D10_300622d-Plate53	
MOR	13	39	20		177	177	H01_230622c-Plate39	
MOR	13	39	50		177	177	H02_230622c-Plate39	
MOR	13	39	100		177	177	H03_230622c-Plate39	
NC-PCR110		53	100		No peaks	No peaks	D04_300622d-Plate53	
RIC	13	53	50		157	157	G09_300622d-Plate53	
RIC	13	53	100		157	157	G10_300622d-Plate53	
SIM	13	53	50		181	181	B11_300622d-Plate53	
SIM	13	53	100		181	181	B12_300622d-Plate53	
SPC	*13*	*39*	*20*		*161*	*177*	*E01_230622c-Plate39*	
SPC	*13*	*39*	*50*		*161*	*177*	*E02_230622c-Plate39*	
SPC	*13*	*39*	*100*	<1 µl added	*No peaks*	*No peaks*	*E03_230622c-Plate39*	
SYR	13	53	50		No peaks	No peaks	H09_300622d-Plate53	
SYR	13	53	100		No peaks	No peaks	H10_300622d-Plate53	
ZIS	*13*	*39*	*20*		169	181	*F01_230622c-Plate39*	*Peaks under 1000 RFU*
ZIS	*13*	*39*	*50*		169	181	*F02_230622c-Plate39*	
ZIS	*13*	*39*	*100*	<1 µl added	*No peaks*	*No peaks*	*F03_230622c-Plate39*	

Table 39. Showing an example of (Ceb11-Plate 3) Dr Tiddi's post Geneious excel file

Ind ID	Plate	Dilution	Name	Ceb11 - 1	Ceb11 - 2
EDU	3	50	A01_GM990_270619	232	232
ERN	3	50	A02_GM990_270619	232	232
TTO	3	50	A03_GM990_270619	232	249
TRU	3	50	A04_GM990_270619	232	232
MAW14	3	50	A05_GM990_270619	234	234
BIA	3	50	A06_GM990_270619	228	228
PAC	3	50	A07_GM990_270619	266	266
MAW11	3	50	A08_GM990_270619	No peaks	No peaks
ING	3	50	A09_GM990_270619	232	232
MAW(H)	3	50	A10_GM990_270619	230	230
BIA(H)	3	50	A11_GM990_270619	222	232
NC	3	50	A12_GM990_270619	No peaks	No peaks
EVA	3	50	B01_GM990_270619	232	232
CL	3	50	B02_GM990_270619	No peaks	No peaks
LIL	3	50	B03_GM990_270619	232	232
ELE	3	50	B04_GM990_270619	No peaks	No peaks
SOL	3	50	B05_GM990_270619	232	232
THE	3	50	B06_GM990_270619	232	232
VEL	3	50	B07_GM990_270619	232	232
BOR	3	50	B08_GM990_270619	228	232
SRP	3	50	B10_GM990_270619	228	232
OCT	3	50	B11_GM990_270619	230	230
M	3	50	B12_GM990_270619	232	232
EDU	3	100	C01_GM990_270619	232	232
ERN	3	100	C02_GM990_270619	232	232
TTO	3	100	C03_GM990_270619	232	249
TRU	3	100	C04_GM990_270619	232	232
MAW14	3	100	C05_GM990_270619	234	234
BIA	3	100	C06_GM990_270619	228	228
PAC	3	100	C07_GM990_270619	266	266
MAW11	3	100	C08_GM990_270619	No peaks	No peaks
ING	3	100	C09_GM990_270619	232	232
MAW(H)	3	100	C10_GM990_270619	230	230
BIA(H)	3	100	C11_GM990_270619	222	232
NC	3	100	C12_GM990_270619	No peaks	No peaks
EVA	3	100	D01_GM990_270619	232	232
CL	3	100	D02_GM990_270619	No peaks	No peaks
LIL	3	100	D03_GM990_270619	232	232
ELE	3	100	D04_GM990_270619	No peaks	No peaks
SOL	3	100	D05_GM990_270619	232	232
THE	3	100	D06_GM990_270619	232	232
VEL	3	100	D07_GM990_270619	232	232
BOR	3	100	D08_GM990_270619	228	232
RIN	3	100	D09_GM990_270619	No peaks	No peaks
SRP	3	100	D10_GM990_270619	228	232
OCT	3	100	D11_GM990_270619	230	230
M	3	100	D12_GM990_270619	232	232

Appendix E. Genotyping Table

Table 40. Showing a sample of the genotyping table, showcasing 2 repeats agreement levels and NC contamination markings

ID name	ID numb	E	Repea	Number o	Ceb03 - 1	Ceb03 - 2	Geneious ID	Comments
ALE	36			2	156	160		
ALE	36	10	E		156	160	F07_290622b-Plate47	
ALE	36	14	E		216	216	D03_010722-Plate57	NC- Peaks at 160 (14832bp)
ANI	37			2	212	212		
ANI	37	9	E		212	212	F07_230622b-Plate38	
ANI	37	13	E		212	212	H03_300622d-Plate53	
AST	1			2	156	160		
AST	1	3	OG		156	160	B03_011019c	
AST	1	7a	E		156	160	C01_310322d-Plate32	
AST	1	7a	P					
BAR	38			2	156	216		
BAR	38	10	E		156	216	B07_290622b-Plate47	
BAR	38	14	E		216	216	H01_010722-Plate57	

Table 41. Showing a sample of the genotyping table, showcasing too many peaks and mismatch designations

ID name	ID numb	E	Repea	Number o	Ceb07 - 1	Ceb07 - 2	Geneious ID	Comments
ALE	36			2	145	153		
ALE	36	10	E		145	145	C09_300622-Plate50	
ALE	36	14	E		145	153	H07_010722b-Plate58	New bin 153
ANI	37			2	125	133		
ANI	37	9	E		125	133	A11_230622d-Plate40	
ANI	37	13	E		No peaks	No peaks	C05_300622e-Plate54	
AST	1			2 (but 1 disco	125	173		
AST	1	3	OG		125	173	F12_GM1046_151019	potential third peak around 130
AST	1	7a	E				**B09_310322d-Plate32**	**Clear/Discounted as 5 believable**
AST	1	7a	P					
BAR	38			2	133/137	133/137		
BAR	38	10	E		137	137	G07_300622-Plate50	dil 50: Clear/Discounted as 3 belie
BAR	38	14	E		133	133	D08_010722b-Plate58	dil 50 has No peaks

Table 42. Key Of Terms And Colours In Genotyping Document

P	Repeats from PCR level
E	Repeats from extraction level
OG	Original sample
	Contaminated NC
	Too many peaks, all discounted
	Mismatching peaks, discounted
	Peaks mismatch
	One clear allele profile vs no peaks
	Two or more allele profiles match

Table 43. Showing final Genotyping Table

s & numb	Ceb02 - 1	Ceb02 - 2	Ceb03 - 1	Ceb03 - 2	Ceb04 - 1	Ceb04 - 2	Ceb07 - 1	Ceb07 - 2	Ceb08 - 1	Ceb08 - 2	Ceb09 - 1	Ceb09 - 2	Ceb10 - 1	Ceb10 - 2	Ceb105 - 1	Ceb105 - 2	Ceb11 - 1	Ceb11 - 2	Ceb115 - 1	Ceb115 - 2	Ceb119 - 1	Ceb119 - 2	Ceb121 - 1	Ceb121 - 2	Ceb127 - 1	Ceb127 - 2	Ceb130 - 1	Ceb130 - 2	D7S794 - 1	D7S794 - 2
ALE36	242	258	156	160	176	184	145	153	169	177	194	198			242	246	232	232	127	135	247	259	140	148	255	255	270	290	126	126
AN37	242	258	212	212	176	184	125	133	177	177	182	194	250	254	238	246	232	232	135	135	247	259	116	136	255	255	194	290	126	142
AST1	214	242	156	160	176	176	125	173	173	177	182	194	254	254	238	242	228	232	135				140	184	255	255	270	274	142	146
BAR38	214	214	156	216	184	188			161	181	194	198			242	246	232	232	127	127	255	259	136	136	255	255	270	274	142	142
BEA39	214	258	212	226	176	176	125	125	177	181	182	194	254	254	238	238	232	232	127	135	243	259	120	140	251	255	270	290	126	126
BIA2	242	258	160	212	176	176	125	137	161	181	182	194	254	254	246	246	232	232	127	131	259	263	120	144	251	251	194	270	126	142
BOR3			156	160	176	176	133	173	169	181	182	182	258	258	246	246	232	232	127	135	263	267	128	132	255	255	270	290	126	142
CAB40	258	258	160	212	176	184	125	125	169	181	182	194			238	246	232	232	127	127					255	255	266	270	142	142
CAM41	242	258	216	226	176	184	125	165	173	181	182	198			238	242	232	232	135	135	255	259	120	140	255	255	270	270	122	142
CLA4	242	242	216	226	176	176	125	125	161	173	182	182	254	254	242	242	232	232	127	135	251	259	160	160		194	270	270	126	146
COR5	242	242	212	212	176	176	125	173	181	181	194	194	254	254	238	242	232	232			255	259			251	251	266	270	142	142
DAL42	242	258	160	212	176	176	125	133			182	194	254	254	238	238	232	232	127	135	259	263	200	200	251	255	194	274	126	126
DAN6	242	242	212	226	176	176	125	137	161	177	182	198	254	254	238	246	232	232	135	135	259	259	176	176	255	255	194	194	126	142
DAT43		212	212	226	176	176	125	161			182	194			246	246			127	127					255	255	194	194	126	126
DAV44	238	238	160	216	176	176	125	161			190	194			242	246	232	232	127	135	259	263	160	160	251	255	226	270	138	142
EDU7	242	242	216	216	176	176	133	133	157	177	182	198	254	254	246	246	232	232	127	127	247	267	132	160		255	270	270	126	146
ELE8	242	242	156	212	176	184		169	169	177					232	232	228	232	135	135	259	259	176	176	255	255	266	270		
ELO45	238	242	160	212	180	184	125	137	161	177	182	198	258	258	242	246	232	232	127	135	259	259	132	132	255	255	194	270	126	142
ERN9	230	230	156	216	176	176	125	125	169	181	182	194	258	258	230	242	228	232	127	127	259	259			255	255	270	270	126	142
ES846	214	242	156	160	176	184	125	173	169	177	194	194	254	254	242	246	232	232	127	135	255	259	140	160	255	255	266	270	126	142
EST10	242	242	160	160	176	176	137	165	169	169	190	194			246	246							128	128						
EVA11	242	242	226	226	176	184	173	173	161	161	158	194	258	258	238	238	232	232	135	135			176	176	255	255	194	274	142	142
FEM47	242	242	212	212	176	176	173	173	181	181	182	198	254	254	238	238	232	232	135	135					255	255			126	142
FRA48	258	258	212	216	176	176			177	177	194	194	254	254	242	246	232	232	127	135	247	259	120	184	255	255	194	290	138	142
GUE49	242	242			176	184	125	141			182	194	254	254	238	242	232	232	135	135	259	263	128	132	255	255	270	270	126	142
GUI50	242	242			176	176	125	125			182	198	250	254											251	255	194	194	126	146
HOR51	242	258	156	216	176	180	125	173	169	181	194	198	250	254	242	246	228	232	127	135	247	259			255	255	270	290	126	146
HUR52	238	238	212	212					169	177	182	186	250	254	238	238	232	232	131	135			116	120	255	255			138	146
ILA53	242	238	160	216	176	180	125	125	157	169	194	198	250	254	242	246	232	232	127	135	259	259	148	148	255	255	270	274	126	146
ING12	242	242	216	216	176	176	125	125	161	173	182	194			242	242	232	232	127	135	259	263			251	255	194	274	126	146
ISI54	242	242	156	216	176	180	125	125			182	194	250	254	242	242	232	232	127	135	259	259	148	148	255	255	194	274	126	146
JAC55			156	160	176	176	125	133			194	198	254	254	242	246	232	232	135	135	247	259	140	172	255	255	194	270	138	142
JC56	258	258	212	216	176	176	125	141	181	181	182	194	254	262	242	242	232	232	135	135	259	259	144	144	255	255	270	270	142	142
JOS13	242	258	212	226	176	176	173	173	161	181	182	194	254	254	242	242	232	232	135	135	259	259			255	255	194	266	138	142
LEN57	242	242	160	216	176	176	125	153			182	194	254	254	226	246	228	228	127	127					255	255	270	270		
LIL14	242	242	212	216	176	184	125	145	169	181	182	194	250	258	246	246	228	232	135	135	259	259	152	172	255	255	270	270	126	142
MAR16	242	242	160	160	176	180	161	169	169	194	194	254	258	258	226	246	232	232	127	127	259	259	120	128	255	255	194	290	138	138
MAT58	214	242	160	216	176	176	125	173	181	181	182	182			238	238	228	232	127	127	259	267	180	180	255	255	270	270	126	142
MAV59					176	176	125	125	157	169					222	234			127	127										
MAW17	242	242	160	212	176	176	125	125	161	181	182	198	258	258	238	246	232	232	127	135	259	263	132	200	251	251	194	270	126	142
MAY18			156	156	176	176					194	194	250	258	226	242	232	232	127	127					255	255	270	270	142	142
MER60	238	238	160	216			137	145	173	177	182	194			242	242	228	232	127	135	247	259	164	164	255	255	290	294	138	138
MGO61	242	242	212	216	176	184	125	137	161	181	182	198	250	254	242	242	232	232	135	135	255	259			251	255	194	270	126	126
MIL62	242	242	156	216	180	184	125	137	157	169	182	198	250	250	246	246	232	232	127	135	259	267			255	255	290	290	126	126
MOI63	214	238	212	226	176	176	125	133	161	177	186	194	250	254	238	246	232	232			243	259			251	255	194	290	142	142
MOR64	242	258	216	216	176	176	125	141	173	177	182	182	250	254	242	242	232	232	127	127	263	267	120	132	251	255	270	274	126	142
MUR65	242	258	160	160	176	184	137	173	177	177	194	194	250	250	238	246	232	232	127	135							266	266	126	126
OCT20			160	160	176	184	125	137	181	181	158	198	250	254	242	246	232	232	135	135	247	259			255	255	266	274	126	142
OFE21	258	258	212	212	176	176	125	173	161	181	182	198	258	258	242	242	232	232							251	255	194	266	126	126
OMA22	242	242			176	176	125	137	177	177	182	194	254	254	242	242	232	232	127	135	247	263	176	176	251	255	270	274	126	126
PAC24	242	242	156	160			137	173			198	198	254	254	242	242	228	232	135	135	247	259	184	184	255	255	266	274	138	142
PIE25	242	242	216	216	176	176	125	125	165	181	182	194	254	254	242	246	228	232	127	135					255	255	270	290	126	146
RIC66							129	173	157	157			254	254			232	232	127	135									126	126
RIN26					180	184	173	173	169	169	182	182	254	254			228	228	135	135	263	263	120	144					126	126
RIT67	242	242	160	216	176	176	125	125	177	177	182	194	254	258	242	246	228	232	135	135	259	259			255	255	266	270	126	146
ROB68	242	258	212	226	176	184	125	133	161	177	194	198	254	254	238	242	232	232	135	135	259	259			255	255	266	270	126	142
SEA69	242	242	156	216	176	180	125	125	161	177	194	198	250	250	246	246			127	127	259	259	120	128	255	255			126	126
SER27			160	216	176	176		125	169	181	194	194	250	258	246	246	228	232	127	135	247	247			255	255	270	290	126	126
SIM70	242	258	216	216	176	176	125	125			178	182			242	242	232	232	127	127	263	267	132	132	251	255	270	274	126	142
SOL28					180	180	137	137			194	194					232	232	131	135									126	126
SPC71	214	258	212	226	176	176	125	137	161	177	186	194	250	254	238	246	232	232	127	135	243	259	120	176	251	255	194	290	142	142
SPO72	214	242	216	226	176	176	125	125	169	181	182	194	250	254	242	246	232	232	127	135	259	259			251	255	194	266	126	142
SRP29	238	242	212	216	176	184	125	125	169	181	182	182	250	254	242	242	232	232	127	127	259	263			255	255	270	274	138	142
SYR73	214	242	212	226			125	137			182	194			238	238	232	232	135	135	231	247	124	124	251	255	194	290	126	138
TET74	242	258	212	216	176	184	125	145	169	177	182	198	254	254	238	238	232	232	135	135			140	140	251	255	266	270	138	138
THE30	242	258	160	160	176	176	125	137	169	181	182	194	258	258	246	246	228	232	127	127	263	263			255	255	266	274	126	142
TRU31	242	242	226	226	172	176	137	161	169	169	194	194			242	242							136	136			194	194	126	126
TTO32	238	242	212	212	176	176	125	125	181	181	182	194	258	258	246	246	232	232	127	127	255	263	156	156	255	255	194	270	142	146
VEL33			216	216	176	176			161	173	190	190	258	258	230	232	232	232	127	135	263	263			255	255				
YOL35	242	242	160	216	176	176	125	137	173	181	182	194	254	254	238	242	228	232	127	135	259	259			255	255	270	274		
ZIS75	242	242	160	216	176	176	125	125	169	181	182	194	254	254	242	246	228	228	127	127	259	263	120	152	255	255	270	274	126	142

Note: Green= Allele profiles with repeat agreement, Black= Allele Profiles without repeat agreement, Mora included

Table 44. Showcasing the Final Genotyping Table after mismatch analysis

ID name & numb	Ceb02 -1	Ceb02 - 2	Ceb03 -1	Ceb03 -2	Ceb04 -1	Ceb04 - 2	Ceb07 -1	Ceb07 - 2	Ceb08 - 1	Ceb08 - 2	Ceb09 -1	Ceb09 - 2	Ceb10 - 1	Ceb10 - 2	Ceb105 - 1	Ceb105 - 2	Ceb11 -1	Ceb11 - 2	Ceb115 - 1	Ceb115 - 2	Ceb119 - 1	Ceb119 - 2	Ceb121 - 1	Ceb121 - 2	Ceb127 - 1	Ceb127 - 2	Ceb130 - 1	Ceb130 - 2	D75794 - 1	D75794 - 2
ALE36	242	258	156	160	176	184	145	153	169	177	194	198	0	0	242	246	232	232	127	135	247	259	140	148	255	255	270	290	126	126
AND7	242	258	212	212	176	184	125	133	177	177	182	194	250	254	238	246	232	232	135	135	247	259	116	136	255	255	194	290	126	142
AST1	214	242	156	160	176	176	125	173	173	177	182	194	254	254	238	242	228	232	127	135	0	0	140	184	255	255	270	274	142	146
BAR38	214	214	156	216	184	188	0	0	161	181	194	198	0	0	242	246	232	232	127	127	255	243	259	136	136	140	251	255	270	274	142	142
BEA39	214	258	212	226	176	176	125	125	177	181	182	194	254	254	238	238	232	232	127	135	259	259	120	140	255	255	270	290	126	126
BIA2	242	258	160	212	176	176	125	137	161	181	182	194	254	254	246	246	232	232	127	131	259	263	120	144	251	251	194	270	126	142
BOR3	0	0	156	160	176	176	133	173	169	181	182	182	258	258	246	246	232	232	127	135	263	267	128	132	255	255	270	290	126	142
CAB40	258	258	160	212	176	184	125	125	169	181	182	194	0	0	238	246	228	232	127	127	0	0	0	0	255	255	266	270	142	142
CAM41	242	258	216	226	176	184	125	165	173	181	182	198	0	0	238	242	232	232	135	135	255	259	120	140	255	255	270	270	122	142
CLA4	242	242	216	226	176	176	125	125	161	173	182	182	254	254	242	242	232	232	135	135	251	259	160	160	0	0	194	270	126	146
COR5	242	242	212	212	176	176	125	173	181	181	194	194	254	254	238	242	232	232	0	0	255	259	0	0	251	251	266	270	142	142
DAI42	242	258	160	212	176	176	125	133	0	0	182	194	0	0	238	238	232	232	127	135	259	263	200	200	251	255	194	274	126	126
DAN6	242	242	212	226	176	176	125	137	161	177	182	198	254	254	238	246	232	232	135	135	259	259	176	176	255	255	194	194	126	142
DAT43	0	0	212	212	176	176	0	0	0	0	182	194	0	0	246	246	0	0	127	127	0	0	0	0	255	255	194	194	126	126
DAV44	238	238	160	216	176	176	125	161	0	0	190	194	0	0	242	246	232	232	127	135	259	263	160	160	251	255	226	270	138	142
EDU7	242	242	216	216	176	176	133	133	157	177	182	198	254	254	246	246	232	232	127	127	247	267	132	160	0	0	270	270	126	146
ELE8	242	242	156	212	176	184	0	0	169	177	0	0	0	0	0	0	232	232	135	135	0	0	176	176	255	255	266	270	0	0
ELO45	238	242	160	212	180	184	125	137	161	177	182	198	0	0	242	246	228	232	127	135	259	259	132	132	255	255	194	270	126	142
ERN9	230	242	156	216	176	176	125	125	169	181	182	194	258	258	230	242	228	232	127	127	259	259	0	0	255	255	270	270	126	142
ESB46	214	242	156	160	176	184	125	173	169	177	194	194	254	254	242	246	232	232	127	135	255	259	140	160	255	255	266	270	126	142
EST10	242	242	242	160	176	176	137	165	169	169	0	0	0	0	246	246	0	0	0	0	0	0	128	128	0	0	0	0	0	0
EVA11	242	242	226	226	176	184	173	173	161	161	158	194	258	258	0	0	232	232	0	0	0	0	176	176	255	255	194	274	142	142
FEN47	242	242	212	212	176	176	173	173	181	181	182	198	254	254	238	238	0	0	135	135	0	0	0	0	255	255	0	0	126	142
FRA48	258	258	212	216	176	176	0	0	177	177	194	194	254	254	242	246	232	232	127	135	247	259	120	184	255	255	194	290	138	142
GUE49	242	242	0	0	176	184	125	141	0	0	182	194	254	254	238	242	232	232	135	135	259	263	128	132	255	255	270	270	126	142
GUE60	242	242	0	0	176	176	125	125	0	0	182	198	250	254	0	0	0	0	0	0	0	0	0	0	251	255	194	270	126	146
HOR51	242	258	156	216	176	180	125	173	169	181	194	198	250	254	242	246	228	232	127	135	247	259	0	0	255	255	290	290	126	146
HUR52	238	238	212	212	0	0	0	0	169	177	182	186	250	254	238	238	232	232	131	135	0	0	116	120	255	255	0	0	138	146
ILA53	242	258	160	216	176	180	125	125	157	169	194	198	250	254	242	246	232	232	127	135	259	259	148	148	255	255	270	274	126	146
ING12	242	242	216	216	176	176	125	125	161	173	182	194	0	0	242	242	232	232	135	135	259	263	0	0	251	255	194	274	126	146
ISI54	242	242	156	216	176	180	125	125	0	0	182	194	250	254	242	242	232	232	127	135	259	259	148	148	255	255	194	270	138	142
JAC55	0	0	156	160	176	176	125	133	0	0	194	198	254	254	242	242	246	232	135	135	247	259	140	144	255	255	0	0	126	142
JC56	258	258	212	216	176	176	125	141	181	181	182	194	0	0	226	242	232	232	135	135	259	259	0	0	255	255	270	270	142	142
JOS13	242	258	212	226	176	176	125	173	161	181	182	194	0	0	242	242	232	232	135	135	259	259	0	0	255	255	194	266	138	142
LEN57	242	242	160	216	176	176	125	153	0	0	182	194	254	254	246	246	228	232	127	127	0	0	0	0	255	255	270	270	0	0
LIL14	242	242	212	216	176	184	125	145	169	181	182	194	250	258	246	246	228	232	135	135	259	259	152	172	255	255	270	270	126	142
MAR16	242	242	160	160	176	180	0	0	161	169	194	194	254	258	226	246	232	232	127	127	0	0	120	128	255	255	194	290	126	138
MAT58	214	242	160	216	176	176	125	173	181	181	182	182	0	0	238	238	228	232	127	127	259	267	180	180	255	255	270	270	126	142
MAV59	0	0	0	0	0	0	125	125	157	169	0	0	0	0	222	234	0	0	127	127	0	0	0	0	0	0	0	0	0	0
MAW17	242	242	160	212	176	176	125	125	161	181	182	198	258	258	238	246	232	232	127	135	259	263	132	200	251	251	194	270	126	142
MAY18	0	0	156	156	176	176	0	0	0	0	194	194	250	258	226	242	232	232	127	135	0	0	0	0	255	255	270	270	142	142
MER60	238	238	160	216	0	0	137	145	173	177	182	194	0	0	242	242	228	232	127	135	247	259	164	164	255	255	290	294	138	138
MGO61	242	242	212	216	176	184	125	137	161	181	182	198	250	254	242	242	232	232	135	135	255	259	0	0	251	255	194	270	126	126
MII62	242	242	156	216	180	184	125	137	157	169	182	198	250	250	246	246	232	232	127	135	259	267	0	0	255	255	290	290	126	126
MOJ63	214	258	212	226	176	176	125	133	161	177	186	194	250	254	238	246	232	232	0	0	243	259	0	0	251	255	194	290	142	142
MOR64	242	258	216	216	176	176	125	141	173	177	182	182	250	254	242	242	232	232	127	127	263	267	120	132	251	255	270	274	126	142
MUR65	242	258	160	160	176	184	137	173	177	177	194	194	250	250	238	246	232	232	127	135	0	0	0	0	255	266	266	266	126	126
OCT20	0	0	160	160	176	184	125	137	181	181	158	198	250	254	242	246	232	232	135	135	247	259	0	0	255	266	266	274	126	142
OFE21	258	258	212	212	176	176	125	173	161	181	182	198	258	258	0	0	232	232	0	0	0	0	0	0	251	255	194	266	126	126
OMA22	242	242	0	0	176	176	125	137	177	177	182	194	254	254	242	242	232	232	127	135	259	263	176	176	251	255	270	274	126	126
PAC24	242	242	156	160	0	0	137	173	0	0	198	198	254	254	242	242	228	232	135	135	247	259	184	184	255	255	266	274	138	142
PIE25	242	242	216	216	176	176	125	125	165	181	182	194	254	254	242	246	228	232	127	135	0	0	0	0	255	255	270	290	126	146
RIC66	0	0	0	0	0	0	129	173	157	157	0	0	254	254	0	0	232	232	127	135	0	0	0	0	0	0	0	0	126	126
RIN26	0	0	0	0	180	184	173	173	169	169	182	182	254	254	0	0	228	228	135	135	263	263	120	144	0	0	0	0	126	126
RIT67	242	242	160	216	176	176	125	125	177	177	182	194	254	258	242	246	228	232	135	135	259	259	0	0	255	255	266	270	126	146
ROB68	242	258	212	212	176	184	125	133	161	177	194	198	254	254	238	242	232	232	135	135	259	259	0	0	255	255	266	270	126	142
SEA69	242	242	156	216	176	180	125	125	161	177	194	198	250	250	246	246	0	0	127	127	259	259	120	128	255	255	0	0	126	126
SER27	0	0	160	216	176	176	0	0	169	181	194	194	250	258	246	246	228	232	127	135	247	247	0	0	255	255	270	290	126	126
SIM70	242	258	216	216	176	176	125	125	0	0	178	182	0	0	242	242	232	232	127	127	263	267	132	132	251	255	270	274	126	142
SOL28	0	0	216	216	0	0	137	137	0	0	194	194	0	0	0	0	232	232	131	135	0	0	0	0	0	0	0	0	126	126
SPC71	214	258	212	226	176	176	125	137	161	177	182	194	250	254	238	246	232	232	127	135	243	259	120	176	251	255	194	290	142	142
SPO72	214	242	242	226	176	176	0	0	177	177	182	194	250	254	242	246	232	232	127	135	259	259	0	0	251	255	194	266	126	142
SRP29	238	242	212	216	176	184	125	125	169	181	182	182	250	254	242	246	232	232	127	135	259	263	0	0	255	255	270	274	138	142
SYR73	214	242	212	226	0	0	125	137	0	0	182	194	250	254	238	238	232	232	135	135	231	247	124	124	251	255	194	290	126	138
TET74	242	258	212	216	176	184	125	145	169	177	182	198	254	254	238	238	232	232	135	135	0	0	140	140	251	255	266	270	138	138
THE30	242	258	160	160	176	176	125	137	169	181	182	194	258	258	246	246	228	232	127	127	263	263	0	0	255	255	266	274	142	142
TRU31	242	242	226	226	172	176	137	161	169	169	194	194	0	0	242	242	0	0	0	0	0	0	136	136	0	0	194	194	126	126
TTO32	238	242	212	212	176	176	125	125	181	181	182	194	258	258	246	246	232	232	127	127	255	263	156	156	255	255	194	270	142	142
VEL33	0	0	216	216	176	176	0	0	161	173	190	190	258	258	230	230	232	232	127	135	263	263	0	0	255	255	0	0	142	146
YOU35	242	242	160	216	176	176	125	137	173	181	182	194	254	254	238	242	228	232	127	135	259	259	0	0	255	255	270	274	0	0
ZS75	242	242	160	216	176	176	125	125	169	181	182	194	254	254	242	246	228	228	127	127	259	263	120	152	255	255	270	274	126	142

Note: Grey background= 0s, Green= fixed mismatches, Orange= remaining mismatches, Mora Included

Appendix F: Cervus Offspring File

Table 10. The Original Offspring File

Offspring ID	Sex	Age Class	Mother ID	Birth Date	Group	Candidate Fathers									
MIL62	M	INFANT	MAY18	2010	GUE	MAR16	CHA77								
DAV44	M	INFANT	RIT67	23/12/2006	GUE	MAR16	CHA77								
HOR51	M	INFANT	ELE8	25/11/2007	GUE	MAR16	CHA77								
JAC55	M	INFANT	RIT67	25/09/2008	GUE	MAR16	CHA77								
ALE36	F	INFANT	ELE8	15/11/2009	GUE	MAR16	CHA77								
ILA53	F	INFANT	RIT67	15/11/2009	GUE	MAR16	CHA77								
ELO45	M	INFANT	ELE8	15/11/2010	GUE	MAR16	CHA77	MER60							
ISI54	M	INFANT	LIL14	15/11/2010	GUE	MAR16	CHA77	MER60							
EDU7	M	INFANT	CAR81	LATE 2006	GUE	MAR16	CHA77								
SEA69	M	INFANT	MAY18	LATE 2008	GUE	MAR16	CHA77								
SER27	M	INFANT			GUE	MAR16	CHA77								
OMA22	M	INFANT	ING12	2010	MAC	ALS76	EDU7	ERN9	HOM78	JES79	JUS80	SER27	TRU31		
LEN57	M	INFANT	THE30	2011	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
ZIS75	M	INFANT	EST10	2011	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
PIE25	M	INFANT	YOL35	2012	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
JC56	M	INFANT	COR5	2013	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
SIM70	M	INFANT	ING12	2013	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
MGO61	M	INFANT	CLA4	09/11/2006	MAC	ALS76	HOM78	JES79	TRU31						
GUI50	F	INFANT	CHI82	08/12/2006	MAC	ALS76	HOM78	JES79	TRU31						
DAL42	M	INFANT	YOL35	04/11/2007	MAC	ALS76	HOM78	JES79	TRU31						
MAV59	M	INFANT	EST10	17/10/2008	MAC	ALS76	HOM78	JES79	JUS80	TRU31	ERN9				
FRA48	M	INFANT	SPO72	01/12/2008	MAC	ALS76	HOM78	JES79	JUS80	TRU31	ERN9				
RIC66	M	INFANT	THE30	25/12/2008	MAC	ALS76	HOM78	JES79	JUS80	TRU31	ERN9				
CAM41	M	INFANT	JOS13	02/11/2009	MAC	ALS76	EDU7	ERN9	HOM78	JES79	JUS80	SER27	TRU31		
FEN47	M	INFANT	YOL35	15/11/2009	MAC	ALS76	EDU7	ERN9	HOM78	JES79	JUS80	SER27	TRU31		
AST1	M	INFANT	YOL35	24/11/2010	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
ESB46	M	INFANT	SOL28	08/02/2011	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
CAB40	M	INFANT	THE30	15/12/2013	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
ROB68	M	INFANT	CLA4	LATE 2009	MAC	ALS76	EDU7	ERN9	HOM78	JES79	JUS80	SER27	TRU31		
MOR64	M	INFANT	ING12	VERY LATE 2010	MAC	EDU7	ERN9	JUS80	SER27	SRP29	TTO32				
SPC71	M	INFANT	SPO72	2010	SPO	ALS76	EDU7	ERN9	HOM78	JES79	JUS80	SER27	TRU31		
ANI37	M	INFANT	DAN6	2012	SPO	RIN26	TRU31								
HUR52	M	INFANT	EVA11	2012	SPO	RIN26	TRU31								
MAT58	M	INFANT	MAW17	2012	SPO	RIN26	TRU31								
MOJ63	M	INFANT	BIA2	2012	SPO	RIN26	TRU31								
MUR65	M	INFANT	SOL28	2012	SPO	RIN26	TRU31								
BEA39	M	INFANT	BIA2	15/11/2010	SPO	TRU31	ERN9	EDU7	JUS80	SER27	SRP29	TTO32	MAR16	CHA77	MER60
DAT43	M	INFANT	DAN6	01/01/2011	SPO	TRU31	ERN9	EDU7	JUS80	SER27	SRP29	TTO32	MAR16	CHA77	MER60
SYR73	M	INFANT	JOS13	01/11/2011	SPO	RIN26	TRU31								

Note: Red= Unsampled Candidate Fathers

Table 45. Final Offspring File input to Cervus

Offspring ID (code and number)	Mother ID	Sex	Age Class	Birth Date	Birth season	Group	Candidate Fathers 1	Candidate Fathers 2	Candidate Fathers 3	Candidate Fathers 4	Candidate Fathers 5
DAV44	RIT67	M	INFANT	23/12/2006	2006	GUE	MAR16	CHA77			
HOR51	ELE8	M	INFANT	25/11/2007	2007	GUE	MAR16	CHA77			
JAC55	RIT67	M	INFANT	25/09/2008	2008	GUE	MAR16	CHA77			
ALE36	ELE8	F	INFANT	15/11/2009	2009	GUE	MAR16	CHA77			
ILA53	RIT67	F	INFANT	15/11/2009	2009	GUE	MAR16	CHA77			
MIL62	MAY18	M	INFANT	2010	2010	GUE	MAR16	CHA77			
ELO45	ELE8	M	INFANT	15/11/2010	2010	GUE	MAR16	CHA77			
ISI54	LIL14	M	INFANT	15/11/2010	2010	GUE	MAR16	CHA77			
OMA22	ING12	M	INFANT	2010	2010	MAC	ERN9	EDU7	SER27	SRP29	TTO32
AST1	YOL35	M	INFANT	24/11/2010	2010	MAC	ERN9	EDU7	SER27	SRP29	TTO32
ESB46	SOL28	M	INFANT	08/02/2011	2010	MAC	ERN9	EDU7	SER27	SRP29	TTO32
MOR64	ING12	M	INFANT	VERY LATE 2010	2010	MAC	ERN9	EDU7	SER27	SRP29	TTO32
LEN57	THE30	M	INFANT	2011	2011	MAC	EDU7	ERN9	SER27	SRP29	TTO32
ZIS75	EST10	M	INFANT	2011	2011	MAC	EDU7	ERN9	SER27	SRP29	TTO32
PIE25	YOL35	M	INFANT	2012	2012	MAC	EDU7	ERN9	SER27	SRP29	TTO32
MAT58	MAW17	M	INFANT	2012	2012	MAC	EDU7	ERN9	SER27	SRP29	TTO32
MUR65	SOL28	M	INFANT	2012	2012	MAC	EDU7	ERN9	SER27	SRP29	TTO32
JC56	COR5	M	INFANT	2013	2013	MAC	EDU7	ERN9	SER27	SRP29	TTO32
SIM70	ING12	M	INFANT	2013	2013	MAC	EDU7	ERN9	SER27	SRP29	TTO32
CAB40	THE30	M	INFANT	15/12/2013	2013	MAC	EDU7	ERN9	SER27	SRP29	TTO32
SPC71	SPO72	M	INFANT	2010	2010	SPO	TRU31	RIN26			
BEA39	BIA2	M	INFANT	15/11/2010	2010	SPO	TRU31	RIN26			
DAT43	DAN6	M	INFANT	01/01/2011	2010	SPO	TRU31	RIN26			
ANI37	DAN6	M	INFANT	2012	2012	SPO	RIN26	TRU31	TET74		
HUR52	EVA11	M	INFANT	2012	2012	SPO	RIN26	TRU31	TET74		
MOJ63	BIA2	M	INFANT	2012	2012	SPO	RIN26	TRU31	TET74		
SYR73	JOS13	M	INFANT	01/11/2011	2011	SPO	RIN26	TRU31			

Note: Red= Unsampled Candidate Fathers

Appendix G. Cervus Outputs

Sample Of The Allele Frequency Analysis Output

Number of individuals: 70
Number of loci: 15
Mean number of alleles per locus: 6.333
Mean proportion of loci typed: 0.8448
Mean expected heterozygosity: 0.6171
Mean polymorphic information content (PIC): 0.5665
Combined non-exclusion probability (first parent): 0.00707180
Combined non-exclusion probability (second parent): 0.00017254
Combined non-exclusion probability (parent pair): 0.00000040
Combined non-exclusion probability (identity): 1.683E-0012
Combined non-exclusion probability (sib identity): 0.00001936

**** Files ****
Input
Genotype data file: Cervus-Genotyping File-no mor (08-11-22).csv
Output
Summary text file: Allele Frequency-Test 1.txt
Allele frequency file: Allele Frequency-Test 1.alf

**** Loci ****
1 Ceb02 -
2 Ceb03 -
3 Ceb04 -
4 Ceb07 -
5 Ceb08 -
6 Ceb09 -
7 Ceb10 -
8 Ceb105 -
9 Ceb11 -
10 Ceb115 -
11 Ceb119 -
12 Ceb121 -
13 Ceb127 -
14 Ceb130 -
15 D7S794 –

Table 46. Showing the Summary Statistics per allele for locus Ceb02

Allele	Count	Hets	Homs	Freq	Freq with null
214	10	8	1	0.0847	0.0775
230	2	0	1	0.0169	0.0083
238	9	3	3	0.0763	0.0510
242	73	21	26	0.6186	0.5291
258	24	16	4	0.2034	0.1822

Number of individuals typed: 59
Heterozygotes: 24
Homozygotes: 35
Number of alleles: 5
Observed heterozygosity: 0.4068
Expected heterozygosity: 0.5674
Polymorphic information content (PIC): 0.5196
Average non-exclusion probability (first parent): 0.8268
Average non-exclusion probability (second parent): 0.6644
Average non-exclusion probability (parent pair): 0.4867

Average non-exclusion probability (identity): 0.2343
Average non-exclusion probability (sib identity): 0.5273
Hardy-Weinberg equilibrium test
Minimum expected frequency: 5.0
Chi-square value (using Yates' correction): 3.0427
Degrees of freedom: 1
P-value: 0.0811
Significance (with Bonferroni correction): NS
Null allele frequency estimate: 0.1520
(Note: File continues by breaking down this information for all the other loci)

The Stimulation Output

Table 47 A & B. Showing the simulated assignment rate at each confidence level for A. Father Alone and B. Father Given Known Mother

Table 47**A. Father Alone**

Level	Confidence (%)	Critical Delta	Assignments	Assignment Rate (%)
Strict	95.00	2.20	4924	49
Relaxed	80.00	0.07	8902	89
Unassigned			1098	11
Total			10000	100

Table 47 **B. Father Given Known Mother**

Level	Confidence (%)	Critical Delta	Assignments	Assignment Rate (%)
Strict	95.00	0.84	7931	79
Relaxed	80.00	0.00	8652	87
Unassigned			1348	13
Total			10000	100

**** Files **** (same as Allele Frequency)
**** Loci ****(same as Allele Frequency)
**** Simulation parameters ****(See Table 5)

Table 48 A & B. Showing the simulated Delta distributions for A. Father Alone and B. Father Given Known Mother

Table 48 **A. Father Alone**

Identity of most likely candidate	N	Mean Delta	Standard Deviation
True father	7175	3.23	2.01
Non-father (true father sampled)	1113	0.94	0.83
Non-father (true father unsampled)	766	1.40	1.10
None	946		
Total	10000		

Table 48 **B. Father Given Known Mother**

Identity of most likely candidate	N	Mean Delta	Standard Deviation
True father	7902	4.92	2.49
Non-father (true father sampled)	395	1.04	1.00
Non-father (true father unsampled)	355	1.74	1.43
None	1348		
Total	10000		

Table 49 A & B. Showing the simulated breakdown of parentage assignments for A. Father Alone and B. Father Given Known Mother

Table 49 **A. Father Alone**

Identity of most likely candidate	Confidence level		
	Strict	Relaxed	Most likely
True father	4678 (95%)	7122 (80%)	7175 (79%)
Non-father (true father sampled)	89 (2%)	1039 (12%)	1113 (12%)
Non-father (true father unsampled)	157 (3%)	741 (8%)	766 (8%)
Total assignments	4924	8902	9054
No assignment made	5076	1098	946
Total tests	10000	10000	10000

Table 49 **B. Father Given Known Mother**

Identity of most likely candidate	Confidence level		
	Strict	Relaxed	Most likely
True father	7535 (95%)	7902 (91%)	7902 (91%)
Non-father (true father sampled)	162 (2%)	395 (5%)	395 (5%)
Non-father (true father unsampled)	234 (3%)	355 (4%)	355 (4%)
Total assignments	7931	8652	8652
No assignment made	2069	1348	1348
Total tests	10000	10000	10000

The Paternity Analysis Outputs

Table 50 A, B & C. Showing the summary statistics of paternity analysis for A. Father Alone (All Offspring), B. Father Alone (Mother Unknown) and C. Father Given Known Mother

Table 50 **A. Father Alone (All Offspring)**

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate (%)	
			Observed	Expected	Observed	Expected
Strict	95.00	0.00	3	27	8	74
Relaxed	80.00	0.00	3	27	8	74
Unassigned			34	10	92	26
Total			37	37	100	100

Table 50 **B. Father Alone (Mother Unknown)**

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate (%)	
			Observed	Expected	Observed	Expected
Strict	95.00	0.00	1	4	20	74
Relaxed	80.00	0.00	1	4	20	74
Unassigned			4	1	80	26
Total			5	5	100	100

Table 50 **C. Father Given Known Mother**

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate (%)	
			Observed	Expected	Observed	Expected
Strict	95.00	0.00	2	23	6	73
Relaxed	80.00	0.00	2	23	6	73
Unassigned			30	9	94	27
Total			32	32	100	100

**** Number of individuals tested ****

Offspring (total): 39
Tested (typed at 8 or more loci): 37

Known mother typed at 8 or more loci: 32
Known mother typed at fewer than 8 loci: 5
Not tested (typed at fewer than 8 loci): 2

Candidate fathers (total): 14
Tested (typed at 8 or more loci): 9
Not tested (typed at fewer than 8 loci): 5
Average number of candidate fathers per offspring: 5
Average proportion of sampled candidate fathers: 0.6626

**** Files ****
Input
Offspring file: Cervus-Offspring File-all infants.csv (includes candidate parent data)
Genotype file: Cervus- Genotyping file- 20-10-22.csv
Allele frequency file: Allele frequency-demo2.alf
Simulation data file: Simulation-demo1.sim
Output
Parentage summary file: Paternity analysis.txt
Parentage data file: Paternity analysis.csv

**** Loci **** (same as Allele Frequency)
**** Simulation parameters ****

Input
Number of offspring: 10000
Number of candidate fathers: 5
Proportion of candidate fathers sampled: 0.7368
Proportion of loci typed: 0.87890000
Proportion of loci mistyped: 0.01000000
Error rate in likelihood calculations: 0.01000000
Minimum number of typed loci: 8
Output
Confidence determined using: Delta
Relaxed confidence level: 80.00%
Strict confidence level: 95.00%

**** Missing genotypes ****
Known mother IDs not found in the genotype file: CAR81, CHI82 (TOTAL: 2)
Candidate father IDs not found in the genotype file: ALS76, CHA77, HOM78, JES79, JUS80, (TOTAL: 5)
**** Excluded individuals ****
The following offspring were excluded from analysis because they were typed at fewer than 8 loci: MAV59 (6 loci), RIC66 (6 loci), (TOTAL: 2)
The following known mothers were excluded from analysis because they were typed at fewer than 8 loci: SOL28 (7 loci) (TOTAL: 1)

Table 51. Showing The Known Mother-Offspring Mismatches For The First Cervus Analysis Run

Locus Name	Offspring ID	Genotype		Known Parent ID	Genotype		Null
Ceb02	MIL62	242	242	MAY18	214	214	Y
Ceb04	MIL62	180	184	MAY18	176	176	
Ceb08	MIL62	157	169	MAY18	177	177	
Ceb09	MIL62	182	198	MAY18	194	194	Y
Ceb10	MIL62	250	250	MAY18	258	258	
Ceb105	MIL62	246	246	MAY18	226	242	
Ceb130	MIL62	290	290	MAY18	270	270	Y
D7S794	MIL62	126	126	MAY18	142	142	Y
Ceb02	DAV44	238	238	RIT67	242	242	Y
Ceb08	DAV44	169	169	RIT67	177	177	Y
D7S794	DAV44	138	142	RIT67	126	146	
Ceb03	HOR51	160	216	ELE8	156	214	
Ceb10	HOR51	250	254	ELE8	258	258	
Ceb119	HOR51	247	259	ELE8	267	267	
Ceb130	HOR51	274	294	ELE8	266	270	
D7S794	HOR51	126	146	ELE8	142	142	
Ceb08	JAC55	157	181	RIT67	177	177	
Ceb09	JAC55	198	198	RIT67	182	194	
Ceb10	JAC55	254	254	RIT67	258	258	Y
Ceb119	JAC55	247	247	RIT67	259	259	Y
Ceb02	ALE36	258	258	ELE8	242	242	
Ceb07	ALE36	145	153	ELE8	125	125	
Ceb119	ALE36	247	259	ELE8	267	267	
Ceb121	ALE36	140	148	ELE8	176	176	
D7S794	ALE36	126	126	ELE8	142	142	
Ceb08	ILA53	157	169	RIT67	177	177	
Ceb10	ILA53	250	254	RIT67	258	258	
Ceb130	ILA53	274	274	RIT67	266	270	
Ceb03	ELO45	160	212	ELE8	156	214	
Ceb08	ELO45	161	177	ELE8	169	181	
Ceb09	ELO45	182	198	ELE8	158	194	
Ceb119	ELO45	259	259	ELE8	267	267	Y
Ceb121	ELO45	132	132	ELE8	176	176	Y
Ceb130	ELO45	194	194	ELE8	266	270	
Ceb10	ISI54	250	254	LIL14	258	258	
Ceb105	ISI54	242	242	LIL14	246	246	Y
Ceb121	ISI54	148	148	LIL14	152	172	
Ceb130	ISI54	194	194	LIL14	270	270	Y
D7S794	ISI54	138	138	LIL14	126	142	
Ceb02	SEA69	242	242	MAY18	214	214	Y
Ceb10	SEA69	250	250	MAY18	258	258	Y
Ceb105	SEA69	246	246	MAY18	226	242	
D7S794	SEA69	126	126	MAY18	142	142	Y
Ceb03	OMA22	218	218	ING12	216	216	Y
Ceb07	OMA22	137	137	ING12	125	125	Y
Ceb08	OMA22	177	177	ING12	161	173	
Ceb10	OMA22	254	254	ING12	258	258	Y
Ceb10	LEN57	254	254	THE30	258	258	Y
Ceb130	LEN57	270	270	THE30	266	274	
Ceb07	ZIS75	125	125	EST10	137	165	
Ceb09	ZIS75	182	194	EST10	146	152	
Ceb121	ZIS75	120	152	EST10	128	128	
Ceb10	PIE25	258	258	YOL35	254	254	Y
Ceb02	JC56	258	258	COR5	242	242	Y
Ceb127	JC56	255	255	COR5	251	251	Y
Ceb08	SIM70	181	181	ING12	161	173	
Ceb10	SIM70	238	242	ING12	258	258	
Ceb10	MGO61	250	254	CLA4	258	258	
Ceb02	FRA48	258	258	SPO72	242	242	Y
Ceb10	FRA48	254	254	SPO72	250	250	Y
D7S794	FRA48	138	142	SPO72	126	126	
Ceb07	CAM41	125	165	JOS13	173	173	
Ceb10	CAM41	254	254	JOS13	258	258	Y
Ceb105	CAM41	238	242	JOS13	226	246	
Ceb130	CAM41	270	270	JOS13	194	266	

D7S794	CAM41	122	126	JOS13	138	142	
Ceb03	FEN47	212	212	YOL35	160	216	
Ceb07	FEN47	173	173	YOL35	125	137	
Ceb09	ROB68	194	198	CLA4	182	182	
Ceb10	ROB68	254	254	CLA4	258	258	Y
Ceb08	MOR64	177	177	ING12	161	173	
Ceb10	MOR64	250	254	ING12	258	258	
Ceb02	SPC71	214	258	SPO72	242	242	
D7S794	SPC71	142	142	SPO72	126	126	Y
Ceb121	ANI37	116	136	DAN6	176	176	
Ceb02	HUR52	238	238	EVA11	242	242	Y
Ceb03	HUR52	212	212	EVA11	226	226	Y
Ceb08	HUR52	169	177	EVA11	161	161	
Ceb09	HUR52	182	186	EVA11	158	194	
Ceb10	HUR52	250	254	EVA11	258	258	
Ceb119	HUR52	247	247	EVA11	259	259	Y
Ceb121	HUR52	116	120	EVA11	176	176	
D7S794	HUR52	138	146	EVA11	142	142	
Ceb121	MAT58	180	180	MAW17	132	200	
Ceb127	MAT58	255	255	MAW17	251	251	Y
Ceb10	MOJ63	250	254	BIA2	258	258	
Ceb121	MOJ63	152	152	BIA2	120	144	
Ceb10	BEA39	254	254	BIA2	258	258	Y
Ceb105	BEA39	238	238	BIA2	246	246	Y
Ceb08	DAT43	189	189	DAN6	161	177	
Ceb10	DAT43	242	246	DAN6	254	254	
Ceb105	DAT43	222	222	DAN6	238	246	
Ceb115	DAT43	127	127	DAN6	135	135	Y
Ceb119	DAT43	235	235	DAN6	259	259	Y
Ceb02	SYR73	214	242	JOS13	258	258	
Ceb07	SYR73	125	137	JOS13	173	173	
Ceb105	SYR73	238	238	JOS13	226	246	
Ceb119	SYR73	231	247	JOS13	259	259	
TOTAL: 98							

Table 52. Showing The Error Rate Analysis For All 15 Markers In The First Cervus Analysis Run

Locus name	N compared	N mismatching	N null	Detection prob.	Est. error rate
Ceb02	30	9	7	0.20993959	0.71449126
Ceb03	32	5	2	0.40237951	0.19415750
Ceb04	30	1	0	0.09533253	0.17482666
Ceb07	26	6	1	0.27763285	0.41560146
Ceb08	28	10	1	0.42991678	0.41536278
Ceb09	32	6	0	0.27090955	0.34605646
Ceb10	25	19	10	0.22991823	1.65276147
Ceb105	28	7	2	0.27829167	0.44916904
Ceb11	28	0	0	0.03476143	0.00000000
Ceb115	29	1	1	0.13637176	0.12642925
Ceb119	24	7	4	0.29964569	0.48668590
Ceb121	9	8	1	0.73837473	0.60192261
Ceb127	29	2	2	0.03957822	0.87125585
Ceb130	27	7	2	0.37465057	0.34600142
D7S794	26	10	4	0.23110849	0.83211003

Mean observed error rate across loci: 0.50845545
(assumes all known parent-offspring pairs are equally independent)

Table 53. Showing the original paternity analysis excel output

Offspring	Loci typed	Mother	Loci typed	Pair loci com	Pair loci mismatch	Pair LOD score	Candidate father	Loci typed	Pair loci com	Pair loci mismatch	Pair LOD score	Pair Delta	Pair con	Trio loci com	Trio loci mismatch	Trio LOD score	Trio Delta	Trio con
MIL62	14	MAY18	12	12	8	-2.94E+01	MAR16	13	12	3	-7.60E+00	0.00E+00		12	10	-6.57E+00	0.00E+00	
DAV44	14	RIT67	14	13	3	-1.23E+01	MAR16	13	12	3	-1.00E+01	0.00E+00		12	7	-1.36E+01	0.00E+00	
HOR51	14	ELE8	14	13	5	-2.08E+01	MAR16	13	12	1	-3.41E+00	0.00E+00		12	7	-8.22E+00	0.00E+00	
JAC55	13	RIT67	14	12	4	-1.59E+01	MAR16	13	11	4	-1.58E+01	0.00E+00		11	7	-1.61E+01	0.00E+00	
ALE36	14	ELE8	14	13	5	-1.84E+01	MAR16	13	12	2	-6.81E+00	0.00E+00		12	5	-5.00E+00	0.00E+00	
ILA53	15	RIT67	14	14	3	-9.41E+00	MAR16	13	13	2	-6.65E+00	0.00E+00		13	6	-8.87E+00	0.00E+00	
ELO45	14	ELE8	14	13	6	-2.32E+01	MER60	13	13	3	-8.49E+00	0.00E+00		13	7	-1.67E-01	0.00E+00	
ISI54	14	LIL14	15	14	5	-1.96E+01	MAR16	13	12	3	-7.59E+00	0.00E+00		12	6	-2.47E-01	0.00E+00	
EDU7	14	CAR81	0	0	0	0.00E+00	MAR16	13	12	5	-1.87E+01	0.00E+00		0	0	0.00E+00	0.00E+00	
SEA69	13	MAY18	12	10	4	-1.26E+01	MAR16	13	11	2	-2.18E+00	0.00E+00		11	6	-2.44E+00	0.00E+00	
SER27	13		0	0	0	0.00E+00	MAR16	13	12	0	2.72E+00	2.72E+00	*	0	0	0.00E+00	0.00E+00	
OMA22	15	ING12	14	14	4	-1.14E+01	ERN9	14	14	5	-1.92E+01	0.00E+00		14	5	-4.95E+00	0.00E+00	
LEN57	11	THE30	14	11	2	-4.99E+00	SER27	13	9	1	-7.63E-03	0.00E+00		9	2	3.01E+00	3.01E+00	*
ZIS75	15	EST10	8	8	3	-8.94E+00	SRP29	14	14	1	-3.26E-01	0.00E+00		14	3	-1.67E-01	0.00E+00	
PIE25	13	YOL35	13	12	1	-2.19E+00	SER27	13	11	0	3.76E+00	3.76E+00	*	11	2	1.29E+00	1.29E+00	*
JC56	15	COR5	13	13	2	-4.00E+00	ERN9	14	14	2	-4.73E+00	0.00E+00		14	4	-3.85E+00	0.00E+00	
SIM70	15	ING12	14	14	2	-4.05E+00	SRP29	14	14	1	-1.33E+00	0.00E+00		14	5	-8.01E+00	0.00E+00	
MGO61	14	CLA4	14	13	1	-1.86E+00	TRU31	10	9	3	-9.20E+00	0.00E+00		9	4	-1.16E+01	0.00E+00	
GUI50	8	CHI82	0	0	0	0.00E+00	TRU31	10	6	2	-6.82E+00	0.00E+00		0	0	0.00E+00	0.00E+00	
DAL42	13	YOL35	13	11	0	2.64E-01	TRU31	10	9	4	-1.44E+01	0.00E+00		9	5	-1.63E+01	0.00E+00	
MAV59	0	EST10	8															
FRA48	15	SPO72	13	13	3	-9.81E+00	ERN9	14	14	4	-1.62E+01	0.00E+00		14	7	-1.70E+01	0.00E+00	
RIC66	0	THE30	14															
CAM41	15	JOS13	14	14	5	-1.77E+01	SER27	13	13	4	-1.47E+01	0.00E+00		13	8	-1.39E+01	0.00E+00	
FEN47	11	YOL35	13	10	2	-4.23E+00	EDU7	14	10	5	-1.78E+01	0.00E+00		10	5	-1.16E+01	0.00E+00	
AST1	14	YOL35	13	12	0	4.34E+00	ERN9	14	13	3	-9.86E+00	0.00E+00		13	4	-1.29E+01	0.00E+00	
ESB46	15	SOL28	0	0	0	0.00E+00	SER27	13	13	2	-6.38E+00	0.00E+00		0	0	0.00E+00	0.00E+00	
CAB40	12	THE30	14	12	0	4.43E+00	TTO32	15	12	1	2.95E-01	2.95E-01	*	12	2	-1.51E+00	0.00E+00	
ROB68	14	CLA4	14	13	2	-7.37E+00	EDU7	14	13	4	-1.31E+01	0.00E+00		13	10	-2.52E+01	0.00E+00	
MOR64	15	ING12	14	14	2	-4.05E+00	EDU7	14	14	2	-2.61E+00	0.00E+00		14	6	-7.94E+00	0.00E+00	
SPC71	15	SPO72	13	13	2	-4.88E+00	SER27	13	13	4	-1.41E+01	0.00E+00		13	7	-2.14E+01	0.00E+00	
ANI37	15	DAN6	15	15	1	-1.33E+00	RIN26	11	11	6	-2.20E+01	0.00E+00		11	8	-2.52E+01	0.00E+00	
HUR52	12	EVA11	13	10	8	-3.10E+01	RIN26	11	9	4	-1.38E+01	0.00E+00		9	8	-4.93E+00	0.00E+00	
MAT58	14	MAW17	15	14	2	-6.50E+00	RIN26	11	10	6	-2.00E+01	0.00E+00		10	6	-1.54E+01	0.00E+00	
MOJ63	15	BIA2	15	15	2	-7.25E+00	TRU31	10	10	6	-2.13E+01	0.00E+00		10	8	-2.50E+01	0.00E+00	
MUR65	12	SOL28	0	0	0	0.00E+00	TRU31	10	9	4	-1.46E+01	0.00E+00		0	0	0.00E+00	0.00E+00	
BEA39	15	BIA2	15	15	2	-6.96E+00	SER27	13	13	4	-1.29E+01	0.00E+00		13	5	-8.24E+00	0.00E+00	
DAT43	11	DAN6	15	11	5	-1.73E+01	TRU31	10	7	3	-9.68E+00	0.00E+00		7	3	-2.82E+00	0.00E+00	
SYR73	13	JOS13	14	12	4	-1.18E+01	TRU31	10	8	2	-4.83E+00	0.00E+00		8	5	-5.51E+00	0.00E+00	

Note: Purple= Offspring With Assigned Fathers

Table 54. Showing mother-offspring mismatches after genotyping table mismatch re-check

Locus Name	Offspring ID	Genotype		Known Parent ID	Genotype		Null
Ceb04	MIL62	180	184	MAY18	176	176	
Ceb09	MIL62	182	198	MAY18	194	194	
Ceb105	MIL62	246	246	MAY18	226	242	
Ceb130	MIL62	290	290	MAY18	270	270	Y
D7S794	MIL62	126	126	MAY18	142	142	Y
Ceb02	DAV44	238	238	RIT67	242	242	Y
D7S794	DAV44	138	142	RIT67	126	146	
Ceb121	ALE36	140	148	ELE8	176	176	
Ceb08	ILA53	157	169	RIT67	177	177	
Ceb121	ELO45	132	132	ELE8	176	176	Y
Ceb105	ISI54	242	242	LIL14	246	246	Y
Ceb121	ISI54	148	148	LIL14	152	172	
Ceb105	SEA69	246	246	MAY18	226	242	
D7S794	SEA69	126	126	MAY18	142	142	Y
Ceb08	OMA22	177	177	ING12	161	173	
Ceb10	LEN57	254	254	THE30	258	258	Y
Ceb130	LEN57	270	270	THE30	266	274	
Ceb02	JC56	258	258	COR5	242	242	Y
Ceb127	JC56	255	255	COR5	251	251	Y
Ceb02	FRA48	258	258	SPO72	214	242	
Ceb130	CAM41	270	270	JOS13	194	266	
Ceb03	FEN47	212	212	YOL35	160	216	
Ceb07	FEN47	173	173	YOL35	125	137	
Ceb09	ROB68	194	198	CLA4	182	182	
Ceb121	ANI37	116	136	DAN6	176	176	
Ceb02	HUR52	238	238	EVA11	242	242	Y
Ceb03	HUR52	212	212	EVA11	226	226	Y
Ceb08	HUR52	169	177	EVA11	161	161	
Ceb09	HUR52	182	186	EVA11	158	194	
Ceb10	HUR52	250	254	EVA11	258	258	
Ceb121	HUR52	116	120	EVA11	176	176	
D7S794	HUR52	138	146	EVA11	142	142	
Ceb121	MAT58	180	180	MAW17	132	200	
Ceb127	MAT58	255	255	MAW17	251	251	Y
Ceb105	BEA39	238	238	BIA2	246	246	Y
Ceb115	DAT43	127	127	DAN6	135	135	Y
Ceb105	SYR73	238	238	JOS13	226	242	
Ceb119	SYR73	231	247	JOS13	259	259	
TOTAL: 38							

Table 55. Showing The Error Rate Analysis For All 15 Markers In The Fixed Mismatches Analysis

Locus name	N compared	N mismatching	N null	Detection prob.	Est. error rate
Ceb02 -	27	4	3	0.17348543	0.42697577
Ceb03 -	30	2	1	0.37365113	0.08920978
Ceb04 -	29	1	0	0.07393893	0.23318405
Ceb07 -	22	1	0	0.27837343	0.08164311
Ceb08 -	21	3	0	0.41902707	0.17046291
Ceb09 -	28	3	0	0.25059659	0.21377557
Ceb10 -	18	2	1	0.17194121	0.32310785
Ceb105 -	27	5	2	0.26386341	0.35091108
Ceb11 -	28	0	0	0.03476143	0.00000000
Ceb115 -	28	1	1	0.13654650	0.13077701
Ceb119 -	19	1	0	0.26739150	0.09841670
Ceb121 -	7	6	1	0.73274908	0.58488157
Ceb127 -	29	2	2	0.03957822	0.87125585
Ceb130 -	27	3	1	0.35535973	0.15633610
D7S794 -	23	4	2	0.23040960	0.37739974

Mean observed error rate across loci: 0.27388914
(assumes all known parent-offspring pairs are equally independent)

Table 56. Showing the paternity analysis excel output with the fixed mismatches

Offspring	Loci typed	Mother	Loci typed	Pair loci com	Pair loci m	Pair LOD s	Candidate	Loci typed	Pair loci com	Pair loci mismatch	Pair LOD score	Pair Delta	Pair con	Trio loci com	Trio loci mismatch	Trio LOD score	Trio Delta	Trio cont
MIL62	14	MAY18	10	10	5	-1.69E+01	MAR16	13	12	3	-7.55E+00	0.00E+00		12	8	-9.79E+00	0.00E+00	
DAV44	13	RIT67	14	12	2	-8.62E+00	MAR16	13	11	3	-1.10E+01	0.00E+00		11	6	-1.41E+01	0.00E+00	
HOR51	14	ELE8	9	8	0	-1.08E+00	MAR16	13	12	1	-3.64E+00	0.00E+00		12	4	-1.18E+01	0.00E+00	
JAC55	12	RIT67	14	11	0	-9.51E-01	MAR16	13	10	2	-8.03E+00	0.00E+00		10	5	-1.81E+01	0.00E+00	
ALE36	14	ELE8	9	9	1	-1.97E+00	MAR16	13	12	1	-3.26E+00	0.00E+00		12	2	-8.15E-01	0.00E+00	
ILA53	15	RIT67	14	14	1	-3.10E+00	MAR16	13	13	2	-6.82E+00	0.00E+00		13	6	-1.46E+01	0.00E+00	
ELO45	14	ELE8	9	9	1	-4.36E+00	MAR16	13	12	2	-6.57E+00	0.00E+00		12	4	-5.97E+00	0.00E+00	
ISI54	14	LIL14	15	14	2	-8.02E+00	MAR16	13	12	3	-9.01E+00	0.00E+00		12	3	8.75E-01	8.75E-01	+
EDU7	14	CAR81	0	0	0	0.00E+00	MAR16	13	12	5	-1.91E+01	0.00E+00		0	0	0.00E+00	0.00E+00	
SEA69	13	MAY18	10	8	2	-4.86E+00	MAR16	13	11	2	-2.26E+00	0.00E+00		11	5	-6.45E+00	0.00E+00	
SER27	12		0	0	0	0.00E+00	MAR16	13	11	0	2.42E+00	2.42E+00	+	0	0	0.00E+00	0.00E+00	
OMA22	14	ING12	13	12	1	1.51E-01	TRU31	10	9	3	-9.18E+00	0.00E+00		9	3	-5.50E+00	0.00E+00	
LEN57	11	THE30	14	11	2	-4.48E+00	SER27	12	9	1	-2.24E-01	0.00E+00		9	2	2.83E+00	2.83E+00	*
ZIS75	15	EST10	0	0	0	0.00E+00	SRP29	14	14	1	-7.70E-01	0.00E+00		0	0	0.00E+00	0.00E+00	
PIE25	13	YOL35	13	12	0	1.97E+00	SER27	12	11	1	-7.82E-01	0.00E+00		11	2	-3.95E+00	0.00E+00	
JC56	15	COR5	13	13	2	-4.58E+00	ERN9	14	14	2	-5.03E+00	0.00E+00		14	4	-4.02E+00	0.00E+00	
SIM70	13	ING12	13	12	0	3.66E+00	SRP29	14	12	0	1.75E+00	1.75E+00	+	12	3	-7.85E+00	0.00E+00	
MGO61	14	CLA4	14	13	0	1.59E+00	TRU31	10	9	3	-9.25E+00	0.00E+00		9	4	-1.16E+01	0.00E+00	
GUI50	8	CHI82	0	0	0	0.00E+00	TRU31	10	6	2	-6.86E+00	0.00E+00		0	0	0.00E+00	0.00E+00	
DAL42	13	YOL35	13	11	0	1.61E-01	TRU31	10	9	4	-1.45E+01	0.00E+00		9	5	-1.70E+01	0.00E+00	
MAV59	0	EST10	0															
FRA48	14	SPO72	13	13	1	-2.61E+00	TRU31	10	9	5	-1.80E+01	0.00E+00		9	6	-1.90E+01	0.00E+00	
RIC66	0	THE30	14															
CAM41	14	JOS13	13	13	1	-3.92E+00	SER27	12	11	4	-1.64E+01	0.00E+00		11	7	-2.15E+01	0.00E+00	
FEN47	11	YOL35	13	10	2	-5.27E+00	SER27	12	9	4	-1.45E+01	0.00E+00		9	4	-1.16E+01	0.00E+00	
AST1	14	YOL35	13	12	0	3.89E+00	ERN9	14	13	3	-1.02E+01	0.00E+00		13	4	-1.30E+01	0.00E+00	
ESB46	15	SOL28	0	0	0	0.00E+00	ERN9	14	14	2	-7.42E+00	0.00E+00		0	0	0.00E+00	0.00E+00	
CAB40	12	THE30	14	12	0	4.99E+00	SRP29	14	12	1	-1.73E+00	0.00E+00		12	2	-3.18E+00	0.00E+00	
ROB68	14	CLA4	14	13	1	-3.32E+00	EDU7	14	13	4	-1.34E+01	0.00E+00		13	9	-2.49E+01	0.00E+00	
MOR64	15	ING12	13	13	0	4.93E+00	EDU7	14	14	2	-3.60E+00	0.00E+00		14	4	-7.82E+00	0.00E+00	
SPC71	15	SPO72	13	13	0	3.68E+00	SER27	12	12	4	-1.50E+01	0.00E+00		12	6	-2.19E+01	0.00E+00	
ANI37	15	DAN6	15	15	1	-1.77E+00	RIN26	10	10	5	-1.82E+01	0.00E+00		10	7	-2.08E+01	0.00E+00	
HUR52	11	EVA11	12	9	7	-2.71E+01	RIN26	10	7	2	-6.15E+00	0.00E+00		7	6	-3.66E+00	0.00E+00	
MAT58	14	MAW17	15	14	2	-6.78E+00	RIN26	10	9	5	-1.61E+01	0.00E+00		9	5	-1.14E+01	0.00E+00	
MOJ63	13	BIA2	15	13	0	8.16E-01	TRU31	10	9	5	-1.75E+01	0.00E+00		9	7	-2.43E+01	0.00E+00	
MUR65	12	SOL28	0	0	0	0.00E+00	RIN26	10	8	4	-1.31E+01	0.00E+00		0	0	0.00E+00	0.00E+00	
BEA39	15	BIA2	15	15	1	-2.69E+00	SER27	12	12	4	-1.45E+01	0.00E+00		12	5	-1.34E+01	0.00E+00	
DAT43	8	DAN6	15	8	1	-1.31E+00	TTO32	15	8	1	6.21E-01	6.21E-01	+	8	2	2.59E-01	2.59E-01	+
SYR73	12	JOS13	13	11	2	-6.58E+00	TRU31	10	8	2	-4.89E+00	0.00E+00		8	4	-7.57E+00	0.00E+00	

Note: Purple= Offspring With Assigned Fathers

Paternity Analysis Output For The 11 Loci

Table 57. Showing summary statistics output for the paternity analysis of the 11 loci test

Father alone (all offspring):

Level	Confidence (%)	Critical Delta	Assignments Observed	Assignments Expected	Assignment Rate Observed	Assignment Rate Expected
Strict	95.00	2.20	5	(13)	19%	(49%)
Relaxed	80.00	0.07	11	(23)	42%	(89%)
Unassigned			15	(3)	58%	(11%)
Total			26	(26)	100%	(100%)

Father alone (mother unknown):

Level	Confidence (%)	Critical Delta	Assignments Observed	Assignments Expected	Assignment Rate Observed	Assignment Rate Expected
Strict	95.00	2.20	1	(0)	100%	(49%)
Relaxed	80.00	0.07	1	(1)	100%	(89%)
Unassigned			0	(0)	0%	(11%)
Total			1	(1)	100%	(100%)

Father given known mother:

Level	Confidence (%)	Critical Delta	Assignments Observed	Assignments Expected	Assignment Rate Observed	Assignment Rate Expected
Strict	95.00	0.84	6	(20)	24%	(79%)
Relaxed	80.00	0.00	7	(22)	28%	(87%)
Unassigned			18	(3)	72%	(13%)
Total			25	(25)	100%	(100%)

**** Number of individuals tested ****

Offspring (total): 26
  Tested (typed at 6 or more loci): 26
    Known mother typed at 6 or more loci: 25
    Known mother typed at fewer than 6 loci: 1
  Not tested (typed at fewer than 6 loci): 0

Candidate fathers (total): 10
  Tested (typed at 6 or more loci): 9
  Not tested (typed at fewer than 6 loci): 1
  Average number of candidate fathers per offspring: 3
  Average proportion of sampled candidate fathers: 0.8462

**** Loci ****
     1 Ceb03 -
     2 Ceb04 -
     3 Ceb07 -
     4 Ceb08 -
     5 Ceb09 -
     6 Ceb11 -
     7 Ceb115 -
     8 Ceb119 -
     9 Ceb127 -
    10 Ceb130 -
    11 D7S794 -

**** Simulation parameters ****
Input
  Number of offspring:10000
  Number of candidate fathers: 10
  Proportion of candidate fathers sampled: 0.8462
  Proportion of loci typed: 0.87400000
  Proportion of loci mistyped: 0.01000000
  Error rate in likelihood calculations: 0.01000000
  Minimum number of typed loci: 6
Output
  Confidence determined using: Delta
  Relaxed confidence level: 80.00%
  Strict confidence level: 95.00%

**** Missing genotypes ****
Candidate father IDs not found in the genotype file: CHA77 (TOTAL: 1)

**** Excluded individuals ****
The following known mothers were excluded from analysis because they were typed at fewer than 6 loci: EST10 (4 loci) (TOTAL: 1)

Table 58. Showing mother-offspring mismatches for the 11 loci output

Locus name	Offspring ID	Genotype		Known parent ID	Genotype		Null
Ceb04 -	MIL62	180	184	MAY18	176	176	
Ceb09 -	MIL62	182	198	MAY18	194	194	
Ceb130 -	MIL62	290	290	MAY18	270	270	Y
D7S794 -	MIL62	126	126	MAY18	142	142	Y
D7S794 -	DAV44	138	142	RIT67	126	146	
Ceb08 -	ILA53	157	169	RIT67	177	177	
Ceb08 -	OMA22	177	177	ING12	161	173	
Ceb130 -	LEN57	270	270	THE30	266	274	
Ceb127 -	JC56	255	255	COR5	251	251	Y
Ceb03 -	ESB46	156	160	SOL28	216	216	
Ceb07 -	ESB46	125	173	SOL28	137	137	
Ceb03 -	HUR52	212	212	EVA11	226	226	Y
Ceb08 -	HUR52	169	177	EVA11	161	161	
Ceb09 -	HUR52	182	186	EVA11	158	194	
D7S794 -	HUR52	138	146	EVA11	142	142	
Ceb127 -	MAT58	255	255	MAW17	251	251	Y
Ceb03 -	MUR65	160	160	SOL28	216	216	Y
Ceb115 -	DAT43	127	127	DAN6	135	135	Y
Ceb119 -	SYR73	231	247	JOS13	259	259	

TOTAL: 19

Table 59. Showing the error rate analysis for the 11 loci output

Locus name	N compared	N mismatching	N null	Detection prob.	Est. error rate
Ceb03 -	24	3	2	0.37617505	0.16614605
Ceb04 -	21	1	0	0.07578904	0.31415523
Ceb07 -	18	1	0	0.27535842	0.10087862
Ceb08 -	15	3	0	0.41564739	0.24058854
Ceb09 -	22	2	0	0.25197211	0.18039515
Ceb11 -	24	0	0	0.03566314	0.00000000
Ceb115 -	22	1	1	0.13672668	0.16622412
Ceb119 -	13	1	0	0.25913156	0.14842476
Ceb127 -	23	2	2	0.03757085	1.15723392
Ceb130 -	21	2	1	0.35548699	0.13395440
D7S794 -	19	3	1	0.23196354	0.34034386

Mean observed error rate across loci: 0.26803133
(assumes all known parent-offspring pairs are equally independent)

Paternity Analysis Output For The 7 Loci

Table 60. Showing summary statistics output for the paternity analysis of the 7 loci test

Father alone (all offspring):

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate	
			Observed	Expected	Observed	Expected
Strict	95.00	2.60	1 (	6)	4%	(24%)
Relaxed	80.00	0.90	8 (	15)	31%	(59%)
Unassigned			18 (	11)	69%	(41%)
Total			26 (	26)	100%	(100%)

Father alone (mother unknown):

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate	
			Observed	Expected	Observed	Expected
Strict	95.00	2.60	0 (	0)	0%	(24%)
Relaxed	80.00	0.90	0 (	1)	0%	(59%)
Unassigned			1 (	0)	100%	(41%)
Total			1 (	1)	100%	(100%)

Father given known mother:

Level	Confidence (%)	Critical Delta	Assignments		Assignment Rate	
			Observed	Expected	Observed	Expected
Strict	95.00	2.10	4 (	12)	16%	(50%)
Relaxed	80.00	0.09	10 (	21)	40%	(86%)
Unassigned			15 (	4)	60%	(14%)
Total			25 (	25)	100%	(100%)

**** Number of individuals tested ****
Offspring (total): 26
Tested (typed at 3 or more loci): 26

Known mother typed at 3 or more loci: 25
Known mother typed at fewer than 3 loci: 1
Not tested (typed at fewer than 3 loci): 0
Candidate fathers (total): 10
Tested (typed at 3 or more loci): 9
Not tested (typed at fewer than 3 loci): 1
Average number of candidate fathers per offspring: 3
Average proportion of sampled candidate fathers: 0.8462

**** Loci ****
1 Ceb04 -
2 Ceb07 -
3 Ceb09 -
4 Ceb115 -
5 Ceb119 -
6 Ceb130 -
7 D7S794 -

**** Simulation parameters ****
Input
Number of offspring: 10000
Number of candidate fathers: 10
Proportion of candidate fathers sampled: 0.8462
Proportion of loci typed: 0.87350000
Proportion of loci mistyped: 0.01000000
Error rate in likelihood calculations: 0.01000000
Minimum number of typed loci: 3
Output
Confidence determined using: Delta
Relaxed confidence level: 80.00%
Strict confidence level: 95.00%

**** Missing genotypes ****
Candidate father IDs not found in the genotype file: CHA77 (TOTAL: 1)
**** Excluded individuals ****
The following known mothers were excluded from analysis because they were typed at fewer than 3 loci: EST10 (2 loci) (TOTAL: 1)

Table 61. Showing mother-offspring mismatches for the 7 loci output

Locus name	Offspring ID	Genotype		Known parent ID	Genotype		Null
Ceb04 -	MIL62	180	184	MAY18	176	176	
Ceb09 -	MIL62	182	198	MAY18	194	194	
Ceb130 -	MIL62	290	290	MAY18	270	270	Y
D7S794 -	MIL62	126	126	MAY18	142	142	Y
D7S794 -	DAV44	138	142	RIT67	126	146	
Ceb130 -	LEN57	270	270	THE30	266	274	
Ceb07 -	ESB46	125	173	SOL28	137	137	
Ceb09 -	HUR52	182	186	EVA11	158	194	
D7S794 -	HUR52	138	146	EVA11	142	142	
Ceb115 -	DAT43	127	127	DAN6	135	135	Y
Ceb119 -	SYR73	231	247	JOS13	259	259	

TOTAL: 11

Table 62. Showing the error rate analysis for the 7 loci output

Locus name	N compared	N mismatching	N null	Detection prob.	Est. error rate
Ceb04 -	21	1	0	0.07578904	0.31415523
Ceb07 -	18	1	0	0.27535842	0.10087862
Ceb09 -	22	2	0	0.25197211	0.18039515
Ceb115 -	22	1	1	0.13672668	0.16622412
Ceb119 -	13	1	0	0.25913156	0.14842476
Ceb130 -	21	2	1	0.35548699	0.13395440
D7S794 -	19	3	1	0.23196354	0.34034386

Mean observed error rate across loci: 0.19776802
(assumes all known parent-offspring pairs are equally independent)

Appendix H: Female Synchrony And Expected Success

Table 63. Showing expected success calculations for Macuco 2012

Cycles Mac 2012	Date	Sub	Number of proceptive females	ERN	SRP	SER	TIT	EDU	
1	10/05/2012	OFE	2	0.5	0.5	0	0	0	
2	13/05/2012	MAW	3	0.33	0.33	0.33	0	0	
3	15/05/2012	YOL	2	0.5	0.5	0	0	0	
4	29/05/2012	MAW	3	0.33	0.33	0.33	0	0	
5	27/05/2012	SOL	3	0.33	0.33	0.33	0	0	
6	29/05/2012	OFE	3	0.33	0.33	0.33	0	0	
7	04/06/2012	ING	1	1	0	0	0	0	
8	09/06/2012	YOL	1	1	0	0	0	0	
9	13/06/2012	THE	1	1	0	0	0	0	
10	01/07/2012	YOL	1	1	0	0	0	0	
11	09/07/2012	SOL	2	0.5	0.5	0	0	0	
12	09/07/2012	OFE	2	0.5	0.5	0	0	0	
13	26/07/2012	YOL	2	0.5	0.5	0	0	0	
14	30/07/2012	THE	2	0.5	0.5	0	0	0	
15	02/08/2012	SOL	2	0.5	0.5	0	0	0	
16	07/08/2012	OFE	2	0.5	0.5	0	0	0	
				9.32	**5.32**	**1.32**	**0**	**0**	**15.96**
				0.58	0.33	0.08	0	0	PROP. ES

Table 64. Showing expected success calculations for Guenon 2011

Cycles Guenon 2011	Date	Sub	Number of proceptive females	MARC	VEL	BAR	MER	OCT		
1	17/06/2011	MAY	1	1	0	0	0	0		
2	02/07/2011	ELE	1	1	0	0	0	0		
3	08/07/2011	MAY	1	1	0	0	0	0		
4	26/07/2011	MAY	1	1	0	0	0	0		
5	20/07/2011	ELE	1	1	0	0	0	0		
6	10/08/2011	ELE	1	1	0	0	0	0		
				6	**0**	**0**	**0**	**0**	1	**EXP (SUC**
				1	0	0	0	0	**1**	PROP ES

Table 65. Showing expected success calculations For Spot 2011

Cycles Spot 2011	Date	Sub	Number of proceptive females	TRU	RIN		
1	29/07/2011	CLA	2	1	1		
2	31/07/2011	SPO	2	1	1		
				0.5	0.5	1	**Exp (Suc)**
				0.5	0.5	1	PROP ES

Table 66. Showing expected success calculations For Spot 2012

Cycles Spot 2012	Date	Sub	Number of proceptive females	TRU	RIN	TET		
	08/06/2012	JOS	1	1	0	0		
	01/07/2012	JOS	1	1	0	0		
	09/07/2012	DAN	1	1	0	0		
				3	**0**	**0**	**3**	**Exp (Suc)**
				1	0	0	1	PROP ES

Appendix I: SPSS

Figure 6. Showing the SPSS GLMM inputs for the PoA (Objective 2)

Appendix J. Statistical Analysis

All GLMMs run had the following warning: The final Hessian matrix is not positive definite although all convergence criteria are satisfied. The procedure continues despite this warning. Subsequent results produced are based on the last iteration. Validity of the model fit is uncertain.

Preliminary Tests

Case Processing Summary

	N	Percent
Included	47	100.0%
Excluded	0	0.0%
Total	47	100.0%

Model Summary

Target		N Of Offspring Sired (per group + year)
Probability Distribution		Binomial
Link Function		Logit
Information Criterion	Akaike Corrected	495.574
	Bayesian	505.376

Information criteria are based on the -2 log likelihood (468.240) and are used to compare models. Models with smaller information criterion values fit better.

Data Structure[a]

	Subjects	Repeated Measures	Target
	Male ID	Year	N Of Offspring Sired (per group + year)
Data for First Subject	CHA77	2006	0
	CHA77	2007	0
	CHA77	2008	0
	CHA77	2009	0
	CHA77	2010	0
Total Number of Levels	11	9	

a. Target: N Of Offspring Sired (per group + year)

Fixed Effects[a]

Source	F	df1	df2	Sig.
Corrected Model	6641864.832	5	41	.000
Rank	6641864.832	5	41	.000

Probability distribution: Binomial
Link function: Logit[a]

a. Target: N Of Offspring Sired (per group + year)

Figure 7. Showing SPSS GLMM preliminary test outputs

Model Summary

Target		N Of Off Sired (per group + year)
Probability Distribution		Binomial
Link Function		Logit
Information Criterion	Akaike Corrected	581.740
	Bayesian	591.542

Information criteria are based on the -2 log likelihood (554.406) and are used to compare models. Models with smaller information criterion values fit better.

Fixed Effects[a]

Source	F	df1	df2	Sig.
Corrected Model	1735832.188	5	41	.000
Rank	1735832.188	5	41	.000

Probability distribution: Binomial
Link function: Logit

a. Target: N Of Off Sired (per group + year)

Note: Case Processing Summary and Data Structure Same as above

Figure 8. Showing SPSS GLMM sensitivity analysis on all betas dataset

Objective 1: Rank & Paternity Success

Case Processing Summary

	N	Percent
Included	26	100.0%
Excluded	0	0.0%
Total	26	100.0%

Model Summary

Target		N Of Offspring Sired (per group + year)
Probability Distribution		Binomial
Link Function		Logit
Information Criterion	Akaike Corrected	688.643
	Bayesian	688.156

Information criteria are based on the -2 log likelihood (670.182) and are used to compare models. Models with smaller information criterion values fit better.

Fixed Effects[a]

Source	F	df1	df2	Sig.
Corrected Model	21.956	5	20	<.001
Rank	21.956	5	20	<.001

Probability distribution: Binomial
Link function: Logit[a]

a. Target: N Of Offspring Sired (per group + year)

Figure 9. Showing GLMM SPSS outputs for Objective 1

Objective 2: PoA and Paternity

Case Processing Summary

	N	Percent
Included	40	100.0%
Excluded	0	0.0%
Total	40	100.0%

Model Summary

Target		N of Offspring Sired
Probability Distribution		Poisson
Link Function		Log
Information Criterion	Akaike Corrected	9981726.663
	Bayesian	9981734.891

Information criteria are based on the -2 log likelihood (9981698.515) and are used to compare models. Models with smaller information criterion values fit better.

Fixed Effects[a]

Source	F	df1	df2	Sig.
Corrected Model	.000	1	38	1.000
ExpectedSuccess	.000	1	38	1.000

Probability distribution: Poisson
Link function: Log[a]

a. Target: N of Offspring Sired

Figure 10. Showing the SPSS Outputs for the PoA GLMM

Table 67. Showing Correlation results for 2010

Correlations[a]

			Expected Success	Observed Success
Kendall's tau_b	Expected Success	Correlation Coefficient	1.000	.739*
		Sig. (2-tailed)	.	.033
		N	8	8
	Observed Success	Correlation Coefficient	.739*	1.000
		Sig. (2-tailed)	.033	.
		N	8	8

*. Correlation is significant at the 0.05 level (2-tailed).

a. Year = 2010

Table 68. Showing Correlation results for 2011

Correlations[a]

			Expected Success	Observed Success
Kendall's tau_b	Expected Success	Correlation Coefficient	1.000	.631
		Sig. (2-tailed)	.	.053
		N	8	8
	Observed Success	Correlation Coefficient	.631	1.000
		Sig. (2-tailed)	.053	.
		N	8	8

a. Year = 2011

Table 69. Showing Correlation results for 2012

Correlations[a]

			Expected Success	Observed Success
Kendall's tau_b	Expected Success	Correlation Coefficient	1.000	-.146
		Sig. (2-tailed)	.	.656
		N	8	8
	Observed Success	Correlation Coefficient	-.146	1.000
		Sig. (2-tailed)	.656	.
		N	8	8

a. Year = 2012

Table 70. Showing Correlation results for 2013

Correlations[a]

			Expected Success	Observed Success
Kendall's tau_b	Expected Success	Correlation Coefficient	1.000	-.055
		Sig. (2-tailed)	.	.866
		N	8	8
	Observed Success	Correlation Coefficient	-.055	1.000
		Sig. (2-tailed)	.866	.
		N	8	8

a. Year = 2013

Objective 3: Infanticide

Table 71. Showing the Squared Deviations Calculations for Male ID

	Squared Deviations							
	2006	2007	2008	2009	2010	2011	2012	2013
CHA77				0.0%	0.0%			
EDU7					1.7%	0.1%	0.1%	0.6%
JUS80					0.6%	0.6%		
RIN26						0.0%		
SER27					14.3%	7.6%	7.6%	3.0%
SRP29					8.7%	2.0%	2.0%	33.3%
TET74							0.0%	
TTO32					1.7%	0.1%	0.1%	0.6%

Table 72. Showing The Van Schaik Calculations from Group-Rank

Sum of Van Schaik	2006	2007	2008	2009	2010	2011	2012	2013	S_Mean
Guenon									
2	0.0%	0.0%	0.0%	30.9%	30.9%	0.0%	0.0%	0.0%	**30.9%**
Macuco									
2	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	10.3%	**18.0%**
3	0.0%	0.0%	0.0%	0.0%	30.9%	-34.6%	-34.6%	10.3%	**-7.0%**
4	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	-56.4%	**1.3%**
5	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	15.4%	10.3%	**18.0%**
6	0.0%	0.0%	0.0%	0.0%	30.9%	15.4%	0.0%	0.0%	**23.2%**
Spot									
2	0.0%	0.0%	0.0%	0.0%	0.0%	30.9%	0.0%	0.0%	**30.9%**
3	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	-100.0%	0.0%	**-100.0%**

Note: Red= will commit infanticide, green= won't commit infanticide

Table 73. Showing Squared deviation for group-rank

	Squared Deviations							
	2006	2007	2008	2009	2010	2011	2012	2013
Guenon								
2				0.0%	0.0%			
Macuco								
2					1.7%	0.1%	0.1%	0.6%
3					14.3%	7.6%	7.6%	3.0%
4					8.7%	2.0%	2.0%	33.3%
5					1.7%	0.1%	0.1%	0.6%
6					0.6%	0.6%		
Spot								
2						0.0%		
3							0.0%	

Table 74. Showing the T Calculations for Group-rank

								95.0%				
	Count	**Degrees o**	**S_VAR**	**S_Std**	**P_Mean**	**S.E**	**t**	**T.Confidence**	**Lower Bou**	**Upper Bou**	**95% POP_CI**	**Commit infanticide**
Guenon												
2	2	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3,0.3]	commit infanticide
Macuco												
2	4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide
3	4	3	10.8%	32.9%	0.0%	16.5%	- 0.42	52.4%	-59.4%	45.4%	[-0.6,0.5]	inconclusive
4	4	3	15.3%	39.2%	0.0%	19.6%	0.07	62.3%	-61.0%	63.7%	[-0.6,0.6]	inconclusive
5	4	3	0.8%	8.9%	0.0%	4.5%	4.04	14.2%	3.8%	32.2%	[0,0.3]	commit infanticide
6	2	1	1.2%	10.9%	0.0%	7.7%	3.00	98.1%	-74.9%	121.3%	[-0.7,1.2]	inconclusive
Spot												
2	1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	30.9%	30.9%	[0.3,0.3]	commit infanticide
3	1	1	0.0%	0.0%	0.0%	0.0%	-	0.0%	-100.0%	-100.0%	[-1,-1]	wont commit infanticide

Note: Purple for rows with only 1 entry so t calculations less reliable, Red 95.0% confidence value used for calculations, Red= will commit infanticide, green= won't commit infanticide, Refer to methods