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Noninvasive paternity assignment in Gombe chimpanzees

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Abstract

The relative success of chimpanzee male mating strategies, the role of male dominance rank and the success of inbreeding avoidance behaviour can only be assessed when paternities are known. We report the probable paternities of 14 chimpanzees included in a long-term behavioural study of chimpanzees (*Pan troglodytes schweinfurthii*) at Gombe National Park, Tanzania. DNA samples were collected noninvasively from shed hair and faeces and genotyped using 13–16 microsatellite loci characterized in humans. All 14 offspring could be assigned to fathers within the community. While there is a positive relationship between male rank and reproductive success, we demonstrate that a range of male mating strategies (possessiveness, opportunistic mating and consortships) can lead to paternity across all male ranks. Several adult females were at risk of breeding with close male relatives. Most successfully avoided close inbreeding but in one case a high-ranking male in the community mated with his mother and produced an offspring. In contrast to recent data on chimpanzees (*P. t. verus*) from the Taï forest, Côte d'Ivoire, no evidence of extra-group paternity was observed in our study. Reanalysis of Taï data using a likelihood approach casts doubt on the occurrence of extra-group paternity in that community as well.

Keywords: chimpanzees, inbreeding, mating strategies, microsatellites, noninvasive sampling, paternity

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Introduction

New methods of noninvasive genotyping to study free-ranging animals offer the potential to characterize reproductive patterns in organisms that are difficult to observe or in species where observed behavioural and genetic mating systems may not be closely correlated (Woodruff 1993; Constable *et al*. 1995; Taberlet *et al*. 1999). An important example is the application of noninvasive genotyping, using shed hair, faeces, or wadges (discarded, chewed food material containing buccal cells) as the DNA source, to examine paternity and mating patterns in wild chimpanzees, *Pan troglodytes* (Morin *et al*. 1994; Gagneux *et al*. 1999), bonobos, *Pan paniscus* (Gerloff *et al*. 1999), and other primate species (e.g. Borries *et al*. 1999). Despite detailed descriptions of social behaviour of chimpanzees over many years at several sites (Ghiglieri 1984; Goodall 1986; Nishida 1990; Wrangham *et al*. 1996), it has proved difficult to characterize the influence of factors such as male dominance, female mate choice, inbreeding avoidance, sperm competition and consortships on male reproductive success. This is because chimpanzees live in complex fission–fusion societies and exhibit variable and promiscuous mating practices. Tests that can assign paternity via genetic exclusion in undisturbed wild chimpanzee populations are therefore critical for better understanding the genetic mating system of our closest living relatives. By adapting and refining currently published methods of DNA extraction from faeces and hair, using a rigorous polymerase chain reaction (PCR) agenda for detecting false homozygotes and spurious band amplification (Taberlet *et al*. 1996; Gagneux *et al*. 1997a) and relying primarily on variable tetra-nucleotide repeat loci, we have generated a reliable picture of paternal identities in one community of Gombe chimpanzees.

Chimpanzees live in communities in which adult males defend a joint territory and have aggressive conflicts with neighbours (Goodall 1986). Male chimpanzees remain in their natal community and establish dominance hierarchies with a clear alpha male, whereas females often transfer

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permanently to new communities at adolescence (Nishida 1979; Goodall 1986; Boesch 1996). Sexually receptive females show conspicuous periovulatory swellings lasting about 13 days in a 36-day cycle and females may mate over 100 times in one cycle, sometimes with all adult males in the community (Tutin 1980). Male chimpanzees follow three different mating patterns: (i) *opportunistic mating*, during which most males mate with the receptive female with no possessiveness shown toward that female; (ii) *possessiveness*, where one male, usually the alpha, maintains close proximity to the female and attempts to maintain exclusive mating access; and (iii) *consortships*, where one male accompanies the female away from the rest of the community, typically for 10–15 days (Tutin 1979; Tutin 1980). Prior to the availability of techniques to determine paternities genetically, it has been difficult to evaluate the success of each pattern.

The most extensive chimpanzee study to date employing noninvasive genetic techniques was carried out in the Taï forest, Côte d'Ivoire (Gagneux *et al*. 1997b, 1999). From the genotypes of 14 infants, their mothers and potential fathers in the group, the authors concluded that as many as half of these infants were not sired by males in the group. It was suggested that another reproductive pattern is for females to make surreptitious visits to other communities to conceive. This was surprising because although females were not continually observed during their conception cycles, visits to mate with extra-community males had never been observed at this site (Gagneux *et al*. 1997b, 1999), although they occasionally occur at Gombe (Goodall 1986).

Gombe National Park, Tanzania, contains three chimpanzee communities (subspecies *P. t. schweinfurthii*), the largest of which, Kasekela, was the focus of our study. The chimpanzees at Kasekela have been studied for 40 years, and were habituated in the early 1960s (Goodall 1986). Gombe National Park (35 km^2) is composed of lowland evergreen forest, increasing elevation semideciduous forest and high elevation grassland. The park is bordered by a rift escarpment (1600 m) to the east and Lake Tanganyika to the west (Collins & McGrew 1988). Observations of chimpanzees are made daily, with presence, reproductive state and social interactions recorded. Since 1975, daily all-day follows of particular individuals have been incorporated into the observation schedule (Goodall 1986). As of May 2000, the Kasekela community consisted of 45 individuals: 11 adult and one adolescent male, 14 adult and two adolescent females and 17 immatures. The current alpha male of the Kasekela community is Frodo, an extremely large (approx. 50 kg) and relatively aggressive individual. Despite many years of studies conducted at Gombe, only two fathers have previously been identified by genetic means (Morin *et al*. 1994).

Over the years, about 50% of adolescent females at Gombe have remained in their natal community, inheriting their mothers' home ranges. These females faced the risk of inbreeding with their fathers and brothers (A. Pusey, J. Schumacher-Stankey & J. Goodall, unpublished results). Any female with adult sons in the group also faces this risk. It has been shown that females actively avoid mating with their male relatives (Goodall 1986; Tutin 1979; Pusey 1980). Males generally show little sexual interest in their mothers or sisters but if they do court them, the females usually resist. Females often avoid sexual overtures by community males old enough to be their fathers, although paternal kin recognition has not been documented in chimpanzees. However, some mating between close relatives does occur (Goodall 1986; A. Pusey, J. Schumacher-Stankey & J. Goodall, unpublished results). Females might avoid inbreeding by engaging in consortships with less closely related males during fertile cycles. Finally, parous females could avoid mating with relatives by conceiving during furtive visits to other communities (Gagneux *et al*. 1999).

The objectives of our study of Gombe chimpanzees were to: (i) improve techniques for noninvasive genotyping and paternity assignment in free-ranging chimpanzees; (ii) assign paternities to examine aspects of chimpanzee breeding behaviour and allow future studies to examine breeding behaviours between paternal relatives; (iii) detect extra-group paternities, should they exist at Gombe, and re-evaluate the evidence for extra-group paternities reported by Gagneux *et al*. (1999) for Taï chimpanzees using a likelihood approach; (iv) compare the relative success of different male mating strategies and the relationship between male rank and reproductive success; and (v) document inbreeding, if any, resulting from matings between mothers and sons or maternally related siblings.

Methods

Hair and/or faecal samples were collected between 1994 and 1999 from as many individuals as possible in the Kasekela community. Male chimpanzees at Gombe usually undergo marked testis enlargement around the age of 9–10 years and first ejaculation is sometimes observed soon afterward (Pusey 1990). Testes do not reach full size until the age of 12–14 years, and it is unlikely that males are fertile before age 10. Samples were obtained from all males aged 10 years and more (18), mothers (seven females), offspring (12), including some 10-year-old males, and six additional females. Four adults with potential fathers in the community were also paternity tested, but only one had a mother in the community that was genotyped. Faecal samples were collected by the staff of the Gombe Stream Research Center. Samples of approximately 2–10 g were placed in individual vials (30 mL to 50 mL size) containing enough 100% ethanol to completely cover the sample (at least a 10:1 ratio of ethanol to faeces), and stored at 4 °C upon arrival in the laboratory. Hair was collected from individual sleeping nests, placed in wax envelopes and

frozen at –20 °C in the laboratory. We do not know the upper limit on viability of ethanol-preserved faecal samples, but our oldest samples (2 years 8 months in ethanol) amplified successfully. Previous samples frozen in liquid nitrogen upon collection and subsequently stored in a –20 °C freezer did not yield DNA after approximately 1 year in storage.

A subset of DNA extractions from faecal material was originally performed following the methods of Gerloff *et al*. (1995), with starting material increased to 100 mg. For the majority of extractions, we followed the methods of Wasser *et al*. (1997) using Qiagen's DNA Tissue Kit, with modifications of increasing the starting material to 100–300 mg, incubating overnight at 60 °C with Qiagen's ATL buffer and Proteinase K only, and a 2-h incubation at 70 °C with Qiagen's AL buffer following the overnight incubation.

Faeces generally formed a slurry at the bottom of the collection vial. Faecal material was pipetted from the slurry with a 1000-μL filter pipette tip (the tapered end cut off with a sterile razor blade to increase the size of the opening). The faecal slurry was added to a 2-mL tube, spun in a microcentifuge for 3 min at top speed, and the top ethanol layer was sucked off with a sterile filter pipette tip and discarded. Each tube was then weighed to estimate the amount of wet faeces. Reagent volumes were then adjusted according to the Qiagen, based on 25-mg increments of weight. After overnight incubation with ATL and proteinase K and the subsequent 2-h incubation with AL, the sample was spun for 5 min at top speed. The supernatant was added to new 2-mL tubes (as many as necessary to accommodate the volume) prior to the addition of ethanol, also added in proportion to the volume of the sample. Spin filtering followed. An individual Qiagen filter can accommodate 650 μL of extract at a time, so the larger volumes that we generated were sequentially added to the spin column until all extract was filtered. Following one wash with AW1 and two washes with AW2, DNA was resuspended in 100 μL Tris EDTA (TE) buffer per 100 mg starting faecal material. Hair was extracted with Chelex following the methods of Morin *et al*. (1994) and Walsh *et al*. (1991) using two to three hairs whenever possible.

Extractions were performed in a germicidal hood with ultraviolet sterilization. All pipetters, tubes and filtered water were sterilized for 15 min with ultraviolet light prior to performing a DNA extraction and the working surface of the hood was washed with a 90% bleach solution. Pipette tips with aerosol-barrier filters were purchased presterilized. The microcentrifuge was located outside the sterile hood.

Faecal DNA was quantified on a Hoefer fluorometer (DyNA Quant 200). Although these measurements would also reflect coextracting bacterial and plant DNA, very low DNA concentrations were not uncommon, indicating very low quantities of coextracting DNA. Fluorometer quantification proved to be very predictive of the success of extracts for subsequent PCR. Samples with less than 5 ng/μL were not used because they rarely worked. Samples yielding at least 5 ng/μL were tested at three different concentrations, 5 ng, 10 ng and 15 ng in a 10-μL PCR reaction, to determine the optimal concentration of DNA for amplification of each sample. This varied by sample depending on the contaminant load coextracting with the DNA. If no amplification occurred, the sample was cleaned using either a re-extraction with the Qiagen kit, or more commonly using the Bio 101 Geneclean Kit with spin filters, following the kit's directions and using fluorometry readings as an estimate of DNA concentration prior to addition of NaI and glassmilk. Following the clean-up procedure, samples were then quantified as before with a fluorometer and again tested to determine the concentration yielding the best amplification. Hair DNA was not quantified on a fluorometer, but was similarly tested at three different concentrations, 1–3 μL of hair extract in a 10-μL PCR reaction. PCR reactions were performed on an MJ Research PTC-100 or a Perkin-Elmer Gene Amp PCR System 9600 using 200-μL thin-walled PCR tubes and a 10-μL PCR reaction mixture, consisting of 10 mM Tris–HCl (pH 9.0), 200 mM $(NH_4)_2SO_4$, 200 μM each dNTP, 3.0 mM $MgCl_2$, 0.5 Units Amplitaq Gold DNA polymerase (Perkin-Elmer), 0.2 μM fluorescently labelled forward primer, 0.2 μM unlabelled reverse primer and DNA concentrations as explained above.

Primers (Research Genetics, Inc) consisted of 15 tetrarepeat loci designed for the human genome (D19S431, D9S905, D18S536, D10S676, D4S1627, D2S1333, D4S243, D1S158, D9S922, D11S1366, D2S1326, D2S433, D20S470, D9S302, D18S851) tested for compatibility in baboons (Phil Morin and colleagues at Sequana Inc., personal communication; Morin *et al*. 1998) and for variability in chimpanzees (Ely *et al*. 1998). We also included one tri-nucleotide repeat human primer (HUMFABP) used with chimpanzees (Morin *et al*. 1994; Gagneux *et al*. 1997a). Reaction conditions were as follows. Initial denaturation of 95 °C for 9–12 min, 11 touchdown cycles (Don *et al*. 1991) of denaturation at 95 °C for 1 min, annealing at 62–68 °C for 1 min and extending at 72 °C for 1 min, decreasing the annealing temperature by 0.5 °C each cycle for 11 cycles, followed by 45 cycles of 95 °C for 1 min, 56–62 °C for 1 min and 72 °C for 1 min. PCR reactions were pipetted in a germicidal hood with ultraviolet sterilization. The entire hood and all contents (pipetters, tubes, racks) were subjected to 15 min of ultraviolet sterilization prior to setting up a PCR reaction. The working surface of the hood was also washed with a 90% bleach solution. We used PCR-dedicated pipetters and aerosol-barrier pipette tips.

Reactions were first visualized on 3% agarose gels stained with ethidium bromide. Successful reactions were then scored on a 6% GenePage Plus acrylamide (Amresco) gel run in an ABI PRISM 373A DNA sequencer (Perkin-Elmer) with an appropriate size standard. Analysis was performed using GENESCAN™ ANALYSIS 2.0 and GENOTYPER™ 1.1 software.

For six offspring, all possible sires within the community (males aged 10 years or more at the conception of the offspring) were genotyped. For another six, all but one possible sire was genotyped and for four adult offspring, fewer possible sires were included because the rest had died before collections began. We retained these latter four in our analysis because a male with a high probability of paternity was identified despite incomplete sampling of males. Given recently described problems associated with noninvasive genetic sampling (Taberlet *et al*. 1999), we took several laboratory and analytical precautions to increase confidence in our genotyping and paternity assignments. To reduce the likelihood of amplification artefacts or 'stutter bands' that could be mistaken as true alleles, we restricted our analysis to tetra- (15 loci) and tri-nucleotide (one locus) repeats, reported to have lower incidence of artefact bands (Edwards *et al*. 1991; Murray *et al*. 1993; Ely *et al*. 1998). We followed a multiple-tubes approach (Gagneux *et al*. 1997a; Taberlet *et al*. 1996) whenever possible, with at least seven independent positive PCR reactions confirming a homozygote and at least three reactions confirming both bands of a heterozygote. Genotypes derived from reactions with fewer reactions are indicated with an asterisk in Table 1 (see also Appendix I). Julie Constable was genotyped at each locus to detect possible human contamination at both the DNA extraction and PCR stage. Faecal and hair-derived DNA for the same individual was used whenever possible for scoring alleles. This allowed a check on the accuracy of faecally derived DNA. Hair and faeces were available for genotyping 19 individuals (underlined genotypes in Table 1), faeces only for 18 individuals and hair only for four individuals (see Appendix I).

Paternity assignment, allele frequencies, observed and expected heterozygosities, tests of Hardy–Weinberg equilibrium and null allele calculations were performed using the computer program CERVUS (Marshall *et al*. 1998). This program calculates paternity inference likelihood ratios and generates a statistic, Δ, defined as the difference in positive log likelihood ratios (LOD) between the top two candidate fathers. If only one candidate father with a positive LOD score exists, his Δ score equals his LOD score. CERVUS uses a simulation based on the observed allele frequencies, taking into account typing error rates and incomplete sampling, to determine the statistical significance of the Δ value generated for each paternity. We used the default error rate of 1%, which represents scoring errors due to such events as misreading a gel, transcribing a number incorrectly, or some error at the level of PCR. We reran the program for each offspring with the exact number of sampled and unsampled males designated. We included all males that were at least 10 years old as possible candidates for paternity (except for Pax who was unable to copulate because of a wound incurred during infancy). We used 100 000 cycles in the simulation to determine the statistical significance of Δ.

We also ran the CERVUS program adding 50% additional unsampled males to account for possible extra-community mating and to determine the effect of many unsampled males on confidence levels of presumed fathers. We calculated paternities using the full genotype set and a genotype set restricted to recommendations of Taberlet *et al*. (1996) using only homozygote genotypes confirmed by at least seven PCR reactions and heterozygote genotpyes confirmed by at least three PCR (Table 1).

CERVUS was chosen over other available methods such as Goodnight & Queller's (1999) KINSHIP program, because of the flexibility of CERVUS in assigning error rate, proportion of possible fathers sampled, proportion of primers used for genotyping and its use of the Δ statistic to determine the most likely paternal candidate. The CERVUS program is also reported to be more robust in discriminating between close relatives as possible fathers (Marshall *et al*. 1998).

Because Gagneux *et al*. (1997b, 1999) concluded that a high rate of extra-group conceptions occurred in the chimpanzees of the Taï forest based on paternity assignments using a strict genetic exclusion approach, we reran their data using the likelihood approach of CERVUS to allow direct comparison with our results from Gombe. For this analysis we used an error rate of 1.5%, based on observed mother–offspring mismatches in the microsatellite data. We first considered only within-group males (sampled and unsampled) as candidates for paternity because they were the most likely fathers based on behavioural data. Given the published results of > 50% extra-group paternities (Gagneux *et al*. 1997b, 1999), we also analysed the data including up to three unsampled extra-group males as paternity candidates.

For our assessment of mating strategies at Gombe, we used a gestation length of 229 days to determine the conception cycle and corresponding mating patterns (Goodall 1986). We also examined mating patterns during any cycle that occurred between 205 and 237 days of the birth to include the range of gestation lengths estimated for this population (Wallis 1997). Demographic data, records of female cycles, copulations and dominance interactions between individuals were recorded by field assistants at the Gombe Stream Research Center under the direction of Dr Jane Goodall. Behaviour is recorded during daily follows on particular focal animals but interactions of note by other group members, for instance copulations or aggressive interactions, are also recorded (Goodall 1986). Behavioural records are translated from Swahili into English, then computerized at the Jane Goodall Institute's Center for Primate Studies at the University of Minnesota under the direction of Dr Anne Pusey.

We used the definitions of Tutin (1980) for describing male mating patterns from the behavioural data. In opportunistic matings, females associated with groups of two to 15 males and no overt sign of competition was noted. Possessiveness was designated if a male showed persistent

Table 1 Genotypes for 39 chimpanzees at 16 loci

Names†	Sex	D19S431	D9S905	D18S536	D10S676	D4S1627	D2S1333	D4S243	D1S548	D9S922	D11S1366	D2S1326	D2S433	HUMFABP	D20S470	D9S302	D18S851
Fanni	F	1/3	3/3	1/9	9/10	1/3	6/7	1/2	3/•*	8/9*	6/8*	4/5	2/4	4/4	3/3	21/21*	3/5*
Fudge-f	M	1/1	3/7	1/6	8/9	3/3	5/7	2/2	2/3*	5/9	8/8*	3/4	2/5	4/4*	3/5	5/21	3/3*
Fifi	F	1/1	3/7	1/9	7/10	1/4	5/6	2/3	2/3*	7/8	6/8	1/4	2/5	4/7	3/4	1/21	3/3*
Fred-h	M	1/12	5/7	1/9	7/7	3/4	1/6	2/3	1/2*	7/8	5/6*	1/2	2/5	4/7*	3/4	1/21	3/3*
Ferdinand-f	M	1/3*	3/5	6/9	7/10	3/4*	6/6*	2/11	2/2	4/8*	6/7	4/5	2/2	4/4	•/•	•/•	•/•
Faustino	M	1/12	3/7	1/6	7/7	3/4	5/6	2/11	1/2*	4/8*	6/8	2/4	4/5	4/4	3/3	1/21	3/3
Gremlin	F	3/12	3/7	1/6	7/9	3/4	1/5	3/11	2/3	4/9	6/7	1/4	5/5	6/6	3/5	21/21	3/5
Gaia-f	F	3/12	3/4*	1/6	7/9	3/4	5/6	11/11	1/3	4/7	7/8	4/5	2/5	4/6	3/4	19/21	3/5
Galahad-f	M	1/3	7/7	1/1	7/9	4/5	5/6	3/11	2/3	4/7	6/19	4/4	2/5	4/6	1/5	21/21	3/7
Sparrow-f	F	1/12	5/7	5/6	7/8*	3/6	1/5*	1/10	3/3*	4/8	7/19	1/3*	4/5	(4/4)	1/5	21/23	3/6
Schweini-f	F	1/12	4/5	4/6	7/8	3/6	5/5*	1/11	3/3	4/•*	6/19	1/2	4/5	4/4*	1/5	19/23	3/4
Jiffy-f	F	12/12*	7/7	1/6	8/9	3/4	1/1*	10/11	2/2*	4/8	6/•*	2/5*	4/6	4/7	3/3*	18/18*	3/5*
Jackson-f	M	1/12	7/7	1/1	8/9	3/4	1/5	10/11	2/2*	4/7*	7/19	1/2	4/5	4/4	3/3	18/21	5/7
Patti	F	1/4	1/7	4/9	8/9	3/6	1/3	1/2	1/1*	7/8*	1/5	1/6	4/5	4/7	1/3	19/21	3/4
Tanga	F	1/12	1/3	6/9	8/9	3/6	1/7*	1/2	1/2*	8/8*	5/8	1/5	4/5	4/7	2/3	21/21*	3/3*
Titan-f	M	1/4	1/5	9/9	9/10	3/3	1/3	2/3	1/2	7/8	5/6	4/6	5/5	4/4*	3/4	19/21	3/3*
Candy-f	F	•/•	•/•	•/•	•/•	4/•*	•/•	1/10	•/•	5/8*	7/8*	1/2	•/•	4/4	3/5*	•/•	3/•*
Conoco-f	F	12/12	1/4	4/6	7/8	3/4	5/6	1/11	1/2	4/8	6/7	1/5	•/•	4/4*	5/5	19/21	3/3
Dilly h	F	1/1*	5/7	1/6	7/9	3/4	6/6*	3/10	1/2	7/8	8/19	1/4	•/•	5/6	3/4	18/21	3/3*
Goblin	M	3/12	1/3	6/9	9/9	3/3	6/7	1/11	2/2	8/9*	5/8*	2/5	4/5	(4/7)	2/5	21/22	3/5
Atlas	M	1/1	5/7	1/9	9/9	3/5	5/6	3/10	2/3*	4/7	6/19	1/4	2/5	4/4	1/3	19/21	3/7
Apollo	M	1/12	1/7	1/1	1/9	3/5	5/6	1/11	1/2	7/7*	19/21	4/5	2/2	4/7	1/1	21/21*	1/6
Beethoven	M	1/1	5/7	1/5	8/9	3/4	5/6	2/3	2/2	7/8	8/19	1/5	5/5	5/7	3/5	7/21	3/3
Freud	M	1/12	5/7	1/5	7/10	1/3	1/6	1/2	1/2*	7/8	8/19	2/4*	1/5	7/7	3/4	1/5	3/3
Frodo	M	1/12	3/5	1/9	7/10	1/3	1/6	2/3	1/2*	7/8*	5/6*	2/4	2/5	4/4	4/4	1/21	3/3*
Prof-f	M	1/7	7/7	1/6	8/9	4/6	1/1	1/3	2/3	5/7	7/7*	2/3	5/5	7/7	3/5	21/21*	3/6
Wilkie	M	6/12	4/7	4/6	7/8	3/6	5/6	3/11	1/3	4/7	6/8	2/5	2/4	4/4	5/5	19/21	3/4
Evered-h	M	3/12	5/7	1/6	7/9	3/4	1/6	3/11	2/3	4/8	6/7	1/5	2/5	4/6	4/5	18/21	3/6
Gimble	M	12/12	3/5	1/6	8/9	1/3*	1/5	3/11	2/3*	8/8*	6/7	1/5	5/5	(4/4)	5/5	21/21*	3/5
Tubi	M	1/12	5/7	1/5	8/9	3/6	1/3	1/3	2/2	1/7	8/9*	2/4	5/1	4/4	3/3	5/21	5/6
Kris	M	12/12	5/7	1/1*	7/9	4/4*	1/6	1/10	2/2	7/8*	8/18	1/4	2/2	5/6	4/5	21/21*	3/6
Sheldon	M	1/4	7/7*	1/6	7/8	3/3*	5/6	1/2	2/3	4/5*	8/•*	3/4*	2/5*	4/4	1/•*	1/•*	3/•*
Mel-f	M	•/•	7/7*	1/4*	9/10*	4/•*	•/•	3/10	2/3	5/•*	•/•	•/•	•/•	(3/3)	•/•	•/•	•/•
Pax	M	1/12	3/5	1/6	7/9	3/4*	1/6	11/11*	•/•	•/•	6/•*	1/4*	5/6	(4/4)	3/4	21/21*	3/6
Aphro-f	F	1/•*	•/•	•/•	8/9*	•/•	•/•	1/2*	•/•	•/•	5/6*	•/•	2/5*	•/•	1/6	21/23*	1/5
Darbee-f	F	1/1*	•/•	•/•	•/•	•/•	•/•	•/•	•/•	•/•	5/•*	•/•	1/2	•/•	3/3*	5/21*	3/•*
Sandi	F	12/12*	7/7	5/9	8/9	3/6*	1/1*	10/10*	1/3*	•/•	20/21	•/•	•/•	(4/7)	4/5	21/21	3/3*
Tita-f	F	1/7	5/5*	•/•	•/•	•/•	•/•	2/9	•/•	•/•	3/5	•/•	5/•*	•/•	3/5	21/22	5/7
Trezia-h	F	1/1*	•/•	•/•	•/•	•/•	•/•	2/3	1/•*	•/•	5/7	•/•	4/5*	•/•	3/3*	2/23	5/6
J. Constable‡		8/9	7/8	**4/5**	**7/9**	–10/–14	–3/2	–5/–4	**3/3**	**1**/–3	**5/6**	**4**/–2	3/3	**6/6**	**1/1**	14/**22**	**3/5**

Underlined genotypes represent those composed of PCR reactions from both hair and faeces.
Genotypes with * represent those confirmed by fewer than seven reactions for a homozygote or fewer than three reactions for a heterozygote.
Genotypes in parentheses for locus HUMFABP were taken from Morin *et al.* (1994).
†'f' & 'h' indicate faeces or hair as only available sample type.
‡Alleles of Julie Constable, responsible for all DNA extractions and genotyping of the Gombe chimpanzees, to detect possible contamination. Alleles shared with the Gombe chimpanzees are in bold.

attention to a female, disrupted copulations and copulation attempts by other males and displayed aggressive behaviour toward males approaching the female. Tutin indicated that such behaviour must occur for at least one hour to be considered possessive. In this study, we considered a male to be possessive if he demonstrated such behaviour at least twice during the female's conception cycle, but most cases involved many more incidences. Our designation of possessiveness therefore may have been less conservative than Tutin's. On the other hand, the data that we were analysing were almost certainly less detailed than Tutin's own data, and subtle possessive behaviour may not always have been recorded. Consortships were determined when a male–female pair were simultaneously absent from the social group for at least 4 days as defined by McGinnis (1979) and Goodall (1986), or were noted as together in the behavioural records. Two females were away from the group during their likely conception cycles and the corresponding genotyped father was also away from the group for some period of time within the female's conception cycle, but the mated pair was not directly observed as being together by any field researchers and the arrival and/or departure dates of the pair were different. These possible consortships are conservatively listed as unknown mating patterns in the data set.

Male dominance was determined by the direction of submissive pant-grunts between pairs (Bygott 1979; de Waal 1982; Goodall 1986). Males were classified as alpha (males that gave no pant-grunts to other males, received pant-grunts from all other males and showed other behaviour typical of alpha males), high-ranking (males that pant-grunted only to the alpha male), middle-ranking (pant-grunted to high-ranking and alpha males), low-ranking (pant-grunted to all but adolescents) and adolescents (at least 10 years old and pant-grunted to all of the above).

Results

Thirty-nine chimpanzees were genotyped and all individuals included in the paternity analysis were scored for 13–16 microsatellite loci (Table 1). The number of alleles per locus ranged from three to 11 and expected heterozygosity (H_E) ranged from 0.556 to 0.841 (Table 2). Tests for deviation from Hardy–Weinberg equilibrium did not reveal significant departures from expected values except for locus D4S1627 where there was heterozygote excess. Given the number of loci tested, at least one test would be expected to return significant results due to a Type I error, even if the null hypothesis were true.

Collections of both hair and faeces from 18 of the chimpanzees provided a confirmation of genotypes at many loci. There were no inconsistencies between faecal and hair DNA in the genotypes reported. Faecal DNA had a higher incidence of allelic dropout and mispriming, the latter producing additional bands much larger or smaller as well as the expected allele bands (Appendix I). Many faecal DNA extractions consistently yielded heterozygote genotypes with no allelic dropout (about 70%), while others had allelic dropout some of the time. Either heterozygote band was a candidate for dropout, but there was a slightly higher incidence of the larger allele of a heterozygote dropping out. With the Qiagen extraction protocol, 71% of extractions yielded DNA that would amplify successfully. Of these successes, 43% required additional cleanup steps (a second Qiagen extraction or GeneClean Kit cleanup, see Methods), and 14% amplified sporadically (25% of the time). Hair extractions successfully amplified more than 90% of the time, except under circumstances when the Chelex matrix was too old and all extractions failed.

Fourteen offspring were candidates for paternity assignment because DNA samples were available from potential fathers resident in the group during the time of their conception (Table 3). Average paternity exclusion probabilities for these offspring were 0.99. Fourteen offspring could be assigned with high confidence (94–99%) to a single male within the community. One additional offspring (Pax) was assigned a father (Evered) with 88% confidence, which agreed with a finding of Morin *et al*. (1994) where both Evered and Goblin were not excluded with the nine loci they tested. Evered was observed on a consortship with Pax's mother.

Although Hardy–Weinberg tests provided no evidence for the presence of null alleles (e.g. significant heterozygote deficiencies), we also applied the CERVUS estimator of null allele frequency which uses an iterative algorithm based on the difference between observed and expected frequency of homozygotes (Summers & Amos 1997). Three loci had estimates of null allele frequencies greater than zero (HUMFABP, D20S470 and D18S851). We analysed our data both with and without these three loci; neither inclusion nor exclusion had any effect on the conclusions drawn about paternity. The only genetic mismatches that occurred between most likely fathers and offspring occurred at locus D20S470. Wilkie had one mismatch with each of two offspring (Faustino and Gaia) and Goblin had one mismatch with one offspring (Fanni) at this locus.

We calculated paternities using the full data set and a data set where only genotypes conforming to the recommendations of Taberlet *et al*. (1996) were used, as explained above (Table 1). Analysis of this restricted data set produced no changes in the identity or statistical confidence of paternity candidates.

To address the issue of extra-community fathers that may have sired an offspring, we reran CERVUS with 50% additional untyped males added to each offspring's analysis. This slightly lowered the Δ values but did not affect the confidence levels for assigned paternities.

Table 2 Alleles, allele frequencies and heterozygosities

Locus	Allele	Frequency	Count	95% Confidence interval	Heterozygosity observed/expected	Null allele estimate
D19S431	1–268	0.4583	33	0.1408	0.667/0.656	−0.0144
	3–276	0.0972	7	0.1482		
	4–280	0.0417	3	0.1408		
	6–288	0.0139	1	0.1360		
	7–292	0.0278	2	0.1385		
	12–312	0.3611	26	0.1521		
D9S905	1–271	0.0857	6	0.1342	0.743/0.723	−0.0154
	3–279	0.1857	13	0.1432		
	4–283	0.0571	4	0.1300		
	5–287	0.2429	17	0.1448		
	7–295	0.4286	30	0.1322		
D18S536	1–141	0.4118	28	0.1114	0.853/0.729	−0.0907
	4–155	0.0735	5	0.1089		
	5–159	0.0735	5	0.1089		
	6–163	0.2647	18	0.1239		
	9–175	0.1765	12	0.1215		
D10S676	1–159	0.0143	1	0.0839	0.886/0.728	−0.1089
	7–183	0.2857	20	0.1140		
	8–187	0.2286	16	0.1144		
	9–191	0.3714	26	0.1073		
	10–195	0.1000	7	0.1033		
D4S1627	1–229	0.0758	5	0.1197	0.818/0.663	−0.1354
	3–237	0.5152	34	0.0984		
	4–241	0.2424	16	0.1336		
	5–245	0.0455	3	0.1140		
	6–249	0.1212	8	0.1262		
D2S1333	1–302	0.3030	20	0.1324	0.818/0.736	−0.0623
	3–310	0.0455	3	0.1140		
	5–318	0.2576	17	0.1336		
	6–322	0.3333	22	0.1306		
	7–326	0.0606	4	0.1170		
D4S243	1–197	0.1974	15	0.1066	0.895/0.810	−0.0579
	2–201	0.2237	17	0.1076		
	3–205	0.2105	16	0.1072		
	9–231	0.0132	1	0.0773		
	10–235	0.1316	10	0.1011		
	11–239	0.2237	17	0.1076		
HUMFABP	3–207	0.0303	2	0.1922	0.394/0.556	0.1736
	4–210	0.6364	42	0.1587		
	5–213	0.0455	3	0.1941		
	6–216	0.1061	7	0.2004		
	7–219	0.1818	12	0.2050		
D2S433	1–180	0.0469	3	0.1325	0.750/0.679	−0.0667
	2–184	0.2813	18	0.1496		
	4–192	0.1719	11	0.1476		
	5–196	0.4688	30	0.1295		
	6–200	0.0313	2	0.1295		
D1S548	1–161	0.2188	14	0.1617	0.688/0.625	−0.0618
	2–165	0.5156	33	0.1334		
	3–169	0.2656	17	0.1620		
D9S922	1–274	0.0161	1	0.0834	0.903/0.749	−0.1049
	4–286	0.2097	13	0.1164		
	5–290	0.0645	4	0.0974		
	7–298	0.2903	18	0.1164		
	8–302	0.3548	22	0.1113		
	9–306	0.0645	4	0.0974		
D11S1366	1–232	0.0152	1	0.0662	0.939/0.841	−0.0650
	3–240	0.0152	1	0.0662		
	5–248	0.1364	9	0.0964		
	6–252	0.2273	15	0.1036		
	7–256	0.1818	12	0.1013		
	8–260	0.2273	15	0.1036		
	9–264	0.0152	1	0.0662		
	18–299	0.0152	1	0.0662		
	19–303	0.1212	8	0.0942		
	20–309	0.0152	1	0.0662		
	21–311	0.0303	2	0.0720		

Table 2 *Continued.*

Locus	Allele	Frequency	Count	95% Confidence interval	Heterozygosity observed/expected	Null allele estimate
D2S1326	1–249	0.2576	17	0.0950	0.970/0.792	−0.1111
	2–253	0.1818	12	0.0922		
	3–257	0.0606	4	0.0697		
	4–261	0.2879	19	0.0942		
	5–265	0.1818	12	0.0922		
	6–269	0.0303	2	0.0585		
D20S470	1–273	0.1111	8	0.1497	0.667/0.726	0.0365
	2–277	0.0278	2	0.1385		
	3–281	0.4167	30	0.1466		
	4–285	0.1667	12	0.1540		
	5–289	0.2639	19	0.1563		
	6–293	0.0139	1	0.1360		
D18S851	1–259	0.0270	2	0.1496	0.595/0.582	0.0019
	3–267	0.6216	46	0.1149		
	4–271	0.0405	3	0.1516		
	5–275	0.1486	11	0.1627		
	6–279	0.1081	8	0.1595		
	7–283	0.0541	4	0.1535		
D9S302	1–211	0.0714	5	0.1436	0.686/0.638	−0.0681
	2–215	0.0143	1	0.1342		
	5–227	0.0571	4	0.1415		
	7–235	0.0143	1	0.1342		
	18–279	0.0714	5	0.1436		
	19–283	0.1000	7	0.1471		
	21–291	0.5857	41	0.1083		
	22–295	0.0286	2	0.1368		
	23–299	0.0571	4	0.1414		

Table 3 Paternity of 14 chimpanzees at Gombe

Offspring	Mother†	Father‡	Paternity exclusion probability	Number of loci	Δ value§	Confidence	Exclusions of 2nd paternity candidate	Sampled/total males aged 10 + years¶
Conoco	Candy	Wilkie	0.99974	16	4.96	99%	1	11/12
Dilly	(Dominie)*	Beethoven	0.98990	16	2.69	96%	1	7/10
Fudge	Fanni*	Sheldon	0.99999	16	7.71	99%	5	13/13
Fanni	Fifi*	Goblin	0.99999	16	7.33	99%	7	7/12
Faustino	Fifi*	Wilkie	0.99976	16	5.55	99%	3	11/12
Ferdinand	Fifi*	Evered	0.99995	13	6.96	99%	4	12/12
Fred	Fifi*	Frodo	0.99963	16	4.58	99%	1	13/13
Gaia	Gremlin*	Wilkie	0.99999	16	5.91	99%	5	13/13
Galahad	Gremlin*	Atlas	0.99999	16	2.16	99%	1	11/12
Gremlin	(Melissa)	Evered	0.99063	16	3.55	94–98%**	N.A.	1/13
Jackson	Jiffy	Atlas	0.99956	16	4.73	99%	3	11/12
Schweini	Sparrow	Wilkie	0.99999	15	9.04	99%	8	11/12
Tanga	Patti	Goblin	0.99987	16	7.03	99%	4	11/12
Titan	Patti	Frodo	0.99988	16	7.10	99%	4	14/14

*Females born in the community.
†(Mother) not typed.
‡Probable fathers shared an appropriate allele (no mismatches) at each locus tested, except for three offspring at locus D20S470, which is suspected to have null alleles. This involved Faustino/Wilkie, Gaia/Wilkie and Fanni/Goblin.
§Δ is calculated by subtracting the two highest positive male LOD scores, and is compared to the simulated Δ to test for significance.
¶Evered was the only paternal candidate with DNA available for Gremlin out of 13 possible males, and this was factored into CERVUS, yet he still had a high probability of paternity.
**98% confidence if first 13 primers analysed. As each additional primer is added, confidence decreases incrementally as LOD decreases then stabilizes at 94% because of a statistical quirk when only one paternal candidate is analysed. See Goodnight & Queller (1999) for a discussion of decreasing likelihood with more loci.

Table 4 Paternity of 14 offspring at Taï

Offspring	Mother	Father	Paternity exclusion probability	Exclusions for likely candidate	Number of loci	Δ value*	Confidence	Sampled/Total adult males
Lychee	Loukoum	Macho	0.999312	0	11	4.27	95%	6/10
Cacao	Castor	Fitz	0.994230	0	9	0.196	99%	9/9
Dorry	Dilly	Kendo	0.999567	0	10	6.12	99%	8/8
Fedora	Fossey	Fitz	0.999994	1	10	0.89	99%	9/9
Gargantua	Goma	Brutus	0.999961	1	10	6.38	99%	9/9
Papot	Perla	Rousseau	0.997740	0	10	2.27	99%	7/7
Vanille	Venus	Kendo†	0.999442	1	11	0.299	99%	9/9
Pandora	Perla	Marius‡	0.999160	4	11	0.133	99%	5/5
Bagheera	Belle	Kendo‡	0.999912	3	9	0.0662	99%	7/7
Congo	Castor	Kendo‡	0.999719	2	11	2.19	99%	9/9
Hector	Hera	Fitz‡	0.999965	3	11	0.667	99%	9/9
Helene	Hera	Gipsy‡	0.999981	2	8	2.70	99%	7/7
Lefkas	Loukoum	Unknown‡	0.999344		8			9/9
Mognie	Mystere	Kendo‡	0.999960	3	10	0.676	99%	9/9

*Δ values calculated assuming an error rate of 1.5% and not including any untyped extra-community males.
†Father originally typed was Ali, with no exclusions. Statistically, Kendo, with one exclusion, was the more likely father, based on the commonness or rarity of paternal alleles shared with the offspring.
‡Paternity was determined to be EGP (extra-group paternity) in the original analysis of Gagneux *et al*. (1997b, 1999).

Our reanalysis of the Taï data yielded a very different view of paternity patterns than that previously reported (Gagneux *et al*. 1997b, 1999). We determined the probability of paternity based on Δ scores for within-group males for the seven offspring described as having been sired by extra-group males. When only within-group males are considered as candidates for paternity all but one offspring had a 99% probability of being fathered by an in-group male despite genetic exclusion at one or more loci for the most likely father (Table 4). Interestingly, the mother of the infant with no in-group assignment was only unobserved for one day, making even this extra-group paternity seem rather unlikely. Paternity probabilities of most likely fathers declined as unsampled 'extra-group' males were added to the analysis, but even with three unsampled males, two potential fathers still had a 95% probability, two had an 85% probability and two had an 80% probability of paternity. If up to half the paternal candidates were listed as unsampled, probabilities declined further.

Table 5 gives the dominance status of Gombe males identified as fathers and the mating strategy leading to the conception. Social status is positively associated with reproductive success for Gombe males. Five of 14 paternities were assigned to alpha males and two to high-ranking males, for a total of 50% of paternities to high-ranking males. There were three confirmed consortships during the period of possible conception, and all three led to paternity by the consort partner. These consortships involved one low- and two middle-ranking males. The low-ranking male was only 13 years old at the time of conception, the youngest male in our study to father an offspring. A fourth consortship corresponded to a paternity with an 88% confidence level (Pax).

Discussion

Analysis of faecal DNA

When we began this study, extraction techniques for faecal DNA were still new and focused on analysis of mitochondrial DNA, which occurs in high copy number in cells. DNA yields were low and many contaminants remained in the extract, often preventing successful PCR amplification of single-copy nuclear loci such as microsatellites. We tested several extraction protocols, beginning with a hexadecyltrimethylammonium bromide (CTAB)-based extraction developed by Tony Goldberg (personal communication) which provided our first successful faecal extractions (Constable *et al*. 1995). This protocol enabled us to extract DNA that was suitable for PCR and gave us an opportunity to develop the most successful PCR criteria. We abandoned the CTAB extraction because the success rate of the other methods was higher and involved less manipulation to achieve extracted DNA, thus reducing the possibility of DNA contamination.

We eventually used modifications of two published extraction protocols. The first was developed for bonobo faeces (Gerloff *et al*. 1995) and represented a modification of a protocol used successfully with bear faeces (Höss *et al*. 1992). The protocol yielded DNA with moderate amounts

Table 5 Father's dominance rank at conception and mating strategy employed

Offspring	Mother	Father	Father's rank at conception	Mating strategies employed by father	Father's age (years)	Father's weight (kg)	Alpha male at conception
Gaia	Gremlin	Wilkie	Alpha	Possessiveness	19	39	Wilkie
Schweini	Sparrow	Wilkie	Alpha	Possessiveness	17	39	Wilkie
Tanga	Patti	Goblin	Alpha	Possessiveness	24	40	Goblin
Conoco	Candy	Wilkie	Alpha	Possessiveness	17	39	Wilkie
Fanni	Fifi	Goblin	Alpha	Opportunistic	16	37	Goblin
Fred	Fifi	Frodo	High	Opportunistic	19	50	Freud§
Titan	Patti	Frodo	High	Opportunistic	17	50	Freud
Faustino	Fifi	Wilkie	Middle	Opportunistic	16	38	Goblin
Ferdinand	Fifi	Evered	Middle	Opportunistic	41	38	Wilkie
Jackson	Jiffy	Atlas	Middle	?*	21	40	Goblin
Galahad	Gremlin	Atlas	Middle	Consortship†	19	36	Goblin§
Gremlin	(Melissa)	Evered	Middle	Consortship†	20	37	Mike
Fudge	Fanni	Sheldon	Low	Consortship†	13	?	Freud§
Dilly	(Dominie)	Beethoven	Low	?‡	16	?	Goblin

*Mating strategy for Jackson's conception is unclear. Possible birthdates for Jackson backdate to encompass two swelling cycles of Jiffy. The first was in the group and opportunistic mating ensued. The second was away from the group, and not observed, however, Atlas was also away from the group for part of this time.

†Consortships were confirmed by simultaneous absence and/or observation.

‡Mating strategy for Dilly's conception is unclear. Possible birthdates backdate to encompass at least two swelling cycles of Dominie. Both occurred away from the group, but Beethoven was also away for much of the same time, and both returned to the group at the same time on three occasions.

§Alpha male is either son or maternal sibling of the mother.

of PCR inhibitors, and by increasing the initial quantity of faeces, produced sufficient concentrations of DNA (20–100 ng/μL). Occasionally a DNA cleanup procedure was needed following the extraction (explained in Methods). The main drawback to the Gerloff *et al*. (1995) protocol was that DNA-binding solution and buffers were not premixed which increased the possibility of introducing contaminant DNA during preparation. We continued the study with the Qiagen DNA Tissue Kit with modifications for bear faeces (Wasser *et al*. 1997). All reagents included in the kit were premixed. Our modifications included increasing the initial quantity of faeces, utilizing higher incubation temperatures and delaying the addition of one incubation reagent. The drawback to the Qiagen protocol was that inhibitors remained, necessitating a further cleaning step for most extractions.

With either extraction technique, a key component to successful DNA amplification was rigorous optimization of the PCR protocol. Our optimized conditions included longer and hotter initial denaturations, a heat-activated *Taq* polymerase (Ampli*Taq* Gold, Perkin-Elmer), a touchdown cycling protocol to utilize the highest possible annealing temperature, increased cycles (> 50), use of thin-walled 200-μl PCR tubes and 10-μl PCR reactions to reach temperatures quickly, and use of minimal amounts of DNA extract (5–15 ng, as measured in a fluorometer) which limited coextracted PCR inhibitors. These PCR modifications proved critical to achieving consistent, scorable PCR products.

We were fortunate to have collections of both hair and faeces from 18 of the chimpanzees, which provided a confirmation of genotypes at many loci. There were no inconsistencies between faecal and hair DNA in the genotypes reported. As indicated in Appendix I, faecal DNA had a higher incidence of allelic dropout and mispriming, especially of bands much larger or smaller than expected at a particular locus, co-occurring with the correct genotype bands. Constable was genotyped at each primer and shared 15 out of 28 possible alleles with the Gombe chimpanzees. Constable's unique alleles appeared as extra (third) bands on seven specific PCR reaction days on one or a few of the chimpanzees' PCR reactions assembled on that day, out of the hundreds of days on which reactions were performed. PCR reactions performed on alternate days for the same individual at the same locus did not show the contaminating bands, demonstrating the importance of performing multiple PCR reactions on different days, especially when the human alleles are in the same size range as those of chimpanzees. We believe contamination by collectors to be negligible because of the consistent matches between hair and faeces (different people collected hair and faeces), and the lack of extra bands in the human range for those loci where humans and chimpanzees have nonoverlapping allele sizes.

Paternity assignment

As a result of our rigorous PCR optimization, we were able to genotype 39 individuals and assign paternity to 14 offspring with confidence (Table 3). One assignment was a confirmation of a previously determined paternity: Wilkie sired Faustino (Morin *et al*. 1994). For three offspring (Dilly, Fanni and Gremlin), paternity was assigned to males that had previously been excluded (Morin *et al*. 1994). Morin's initial genetic study on the Gombe chimpanzees was conducted before full realization of the problems of allelic dropout and spurious band amplification when working with extremely low concentrations of DNA (Taberlet *et al*. 1996; Gagneux *et al*. 1997a; Goossens *et al*. 1998; Taberlet *et al*. 1999). The three paternity exclusions in Morin's study that we assigned could be attributed to false homozygosity in the offspring, mother, or the excluded male candidate, as well as to scoring errors (Appendix II).

Our reassignment of Evered as the sire of Gremlin is strengthened by behavioural data indicating that Evered engaged in a lengthy consortship with Melissa, Gremlin's mother (Goodall 1986). Although Evered was the only sampled paternity candidate for Gremlin (all other candidate males died and samples were not collected), he had a high likelihood of paternity. Evered and Gremlin shared an allele at each of 16 loci, plus an additional two unreported loci. As the sole paternity candidate, there was an interesting statistical effect on the confidence of Evered's Δ. Increased loci added to his genotype yielded decreasing LOD (Goodnight & Queller 1999) and thus decreasing Δ. Evered's confidence decreased from 98% to 94% with an increase in loci from 13 to 16 (Table 3) and no mismatches.

Mating within the community

Our results agree with the traditional view that chimpanzee social units generally correspond to reproductive units. Although there have been a few observations of female chimpanzees copulating with males outside their community at Gombe, all offspring tested in our sample were linked with high probability (94–99%) to a candidate father within the community. At Gombe, extra-group paternity might be expected because the risk of inbreeding is quite high; over half the offspring typed at Gombe were born to natal females having adult male relatives in the community. This was not the case, however, as demonstrated by the high within-group paternity. Natal females had a choice of 10–11 males that were neither fathers nor first order maternal relatives in the community to mate with (depending on the conception date of their offspring) In bonobos, extra-group matings have been observed, even in the presence of community males. Nevertheless, definitive paternity assignments in bonobos were to within-group males (Gerloff *et al*. 1999).

The low incidence of extra-group paternity for bonobos and Gombe chimpanzees and the relatively high incidence reported among Taï chimpanzees (Gagneux *et al*. 1997b; Gagneux *et al*. 1999) led us to re-examine the analysis of Gagneux *et al*. using CERVUS. Our reanalysis casts some doubt on the incidence of extra-group paternity at Taï. When only community males are included, CERVUS assigned paternity to a community male in all but one case. Inclusion of unsampled males lowers statistical confidence for most likely fathers. Clearly, the number of unsampled males included in the analysis is critical for likelihood-based paternity analysis.

Decisions regarding unsampled males as potential fathers can be guided by behavioural observations. For instance, the length of time a female is away from her community during the last weeks of swelling, when she is most likely to get pregnant would be one indicator of possible copulations with extra-community males, as Gagneux *et al*. suggest. However, the female was absent for long periods during several of the conceptions in our study, and the father proved to be a community male who was also absent for some of the period. This possibility should be considered before concluding that the female was not mating with community males.

The challenges and limitations of noninvasive genetic typing should also be considered when unexpected results occur (Taberlet *et al*. 1999). When genetic exclusions occur for paternal candidates identified by likelihood analysis, the genetic data should be rigorously re-examined and retested to be certain that no genotyping errors have occurred. Use of tri- and tetra-nucleotide microsatellites lowers the occurrence of stutter bands and reduces ambiguity in scoring allele sizes. Loci should be chosen with high levels of heterozygosity and minimal overlap in allele sizes between humans and chimpanzees to more readily detect contamination by human DNA. More accurate scoring can be achieved with the use of automated sequencing technology and the inclusion of labelled size standards in each lane. Production of electropherograms with automated sequencing software quantifies band intensity and provides better criteria for distinguishing true alleles from artefacts. Finally, PCR reactions for each individual at each locus should be repeated at different times to confirm genotypes (Appendix I).

It is certainly possible that mating patterns between Taï and Gombe are indeed different due to intraspecific behavioural variation and/or local ecological and demographic conditions. The question of the prevalence of extra-group conceptions in chimpanzees will only be fully resolved when extra-community males are also genotyped. At Gombe we have begun to genotype the adjoining Mitumba community and collections have begun at the neighbouring Kalande community. Further behavioural and genetic studies at Taï as well as other chimpanzee communities can shed more light on this issue.

Success of male mating strategies and the role of male dominance rank

All three mating strategies, possessiveness (or mate guarding), opportunistic and consortships, led to conceptions (Table 5). In four of the 12 conceptions for which mating patterns where known, the alpha male was possessive and achieved paternity. In a fifth case (the conception of Ferdinand), the alpha male was also possessive (Constable 2000), but another male succeeded in becoming the father by mating in an opportunistic fashion (Table 5). In four more conceptions, the father also mated opportunistically. In one of these (Fanni's), the father was also the alpha male, and in two other cases, the alpha male was not guarding the female, either because he was engaged with guarding another swollen female (Faustino's conception), or because he was a close relative of the mother (Fred's conception) (Constable 2000). Finally, in three conceptions, the fathers were known to be in consortship with the female (Table 5). Bonobos have also been observed to practice different mating patterns (Kano 1989; Gerloff *et al*. 1999). Success of possessive behaviour by dominant males has been demonstrated by paternity assignment (Gerloff *et al*. 1999), but paternity associated with other strategies (opportunistic mating and consortships) has not been shown elsewhere.

Dominance rank is correlated with reproductive success and influences the mating strategy that is used by an individual. Alpha males accounted for 36% of all conceptions in our study. However, if we exclude three conceptions that occurred when a close male relative (son or brother) of the cycling female was alpha and thus would not be expected to compete for her (see below), alpha males were responsible for 45% of conceptions, and high-ranking males for 50%. Over the 20 years of our study, the average number of high-ranking (including alpha) males per year was 3.35, with a low of two males per year and a high of five males per year. The importance of high rank to reproductive success has been demonstrated in other primates, including red howler monkeys, *Alouatta seniculus* (Pope 1990), long-tailed macaques, *Macaca fascicularis* (de Ruiter *et al*. 1994), stump-tailed macaques, *Macaca arctoides* (Bauers & Hearn 1994), savanna baboons, *Papio cynocephalus* (Altmann *et al*. 1996), and bonobos (Gerloff *et al*. 1999). Among Taï forest chimpanzees, Gagneux *et al*. (1999) report that alpha males sired only 21% of offspring, and dominant males (alpha and ex-alpha) were found to sire 36% of offspring. However, our reanalysis of the Taï data using CERVUS (Marshall *et al*. 1998) increases dominant male paternities to a possible high of 71%.

While only alpha males practiced possessiveness in this sample of mating cycles leading to conception, both high- and middle-ranking males achieved paternity through opportunistic mating. With 57% of conceptions resulting from this pattern, it was the most common strategy overall. The majority of copulations observed at Gombe are opportunistic (Tutin 1980) but previously Tutin questioned the success rate of opportunistic mating in achieving paternity because most of the conceptions she studied occurred in association with possessive behaviour (mate-guarding) or consortships. Genetic confirmations of paternity were not performed, however, and are clearly critical for accurate assessment of genetic mating systems in chimpanzees. In our study, even in cycles in which the alpha male was possessive, the female always managed to mate with other males during her swelling (Constable 2000). This and the fact that in many fertile cycles there may be little effective possessive behaviour, confirm the great opportunity for sperm competition in this species (Harcourt *et al*. 1981; Hasegawa & Hiraiwa-Hasegawa 1990).

Middle- and low-ranking males practice opportunistic mating, but may have a much greater chance of success if they can form a consortship with a female. All three consortships during conception cycles in this study led to a paternity and provided the only cases of successful paternity by low-ranking males. Consortships were associated with at least 21% of the offspring we typed. Our findings agree with Gombe records from 1975 to 1994 where 25% of all conceptions were associated with consortships (Wallis 1997). Paternity assignment to male consorts confirms the previously inferred success of consortships, although it may represent a difficult strategy. Females can exercise some choice with this option; if the consort is not desired they can often alert nearby community males to rescue them. This strategy also poses a risk because moving to the edge of the territory away from other community members could expose consorting pairs to attacks from neighbouring communities.

Inbreeding avoidance

Females born at Gombe that reach adolescence often face the potential of inbreeding with close relatives, either brothers or fathers, and at a later age with sons. Females might avoid the risks of inbreeding by removing themselves from relatives during fertile cycles. Most or all adolescent females born at Gombe visit other communities early in adolescence (Pusey *et al*. 1997), and some may conceive their first offspring in this manner (Pusey 1980; Goodall 1986). But unlike at other sites (Boesch 1997; Nishida *et al*. 1990), 50% return and remain in their natal community. Mating usually occurs at only low rates, if at all, between these females and their close relatives, both because the males are often not interested in mating with their relatives and because females usually resist their attempts (Tutin 1979; Pusey 1980; Goodall 1986; A. Pusey, J. Schumacher-Stankey & J. Goodall, unpublished results). This is also the case in other primate species (reviewed in Pusey 1990). Nevertheless, some closely related pairs do mate at Gombe (Goodall 1986; A. Pusey,

J. Schumacher-Stankey & J. Goodall, unpublished results), and another way to avoid a high-ranking male relative is to engage in a consortship with an unrelated male. This strategy was employed by the natal females Fanni and Gremlin when they had high-ranking male relatives. During Fanni's conception of Fudge, she was never observed mating with her probable father, Goblin, or her two brothers Freud (alpha) and Frodo. Rather, she conceived during a consortship with low-ranking Sheldon. Gremlin conceived twice while her probable father, Evered, and brothers, Goblin and Gimble, were in the community and she was observed to mate with all these males between 1975 and 1997 (A. Pusey, J. Schumacher-Stankey & J. Goodall, unpublished results). During Goblin's tenure as alpha male, however, she was on consortship for most of her conception cycle with the mid-ranking male, Atlas, who fathered Galahad. For her second conception (Gaia), an unrelated alpha male, Wilkie, guarded her and fathered her offspring.

Our findings suggest that females are usually able to avoid inbreeding even in the presence of relatives, but this is not infallible. Fifi, a natal female has had eight infants and for most of her reproductive career has had either maternal brothers, or adult sons in the community. Her first infant was conceived in another group, the second, during a consortship with an unrelated male, and the rest during mating in the group. Four of these six were genotyped in this study, and Fifi successfully avoided her close relatives for three of these conceptions. However, during the conception of her seventh infant, her oldest son, Freud, the alpha male, showed no interest in her but her second son, Frodo, was observed attempting to copulate with her and he turned out to be the father of her infant, Fred. Frodo has been witnessed forcefully completing copulations with Fifi on other occasions. He is the largest chimpanzee on record in this group, and is particularly aggressive, and therefore this conception may prove an exceptional case. Interestingly, Fred is the only one of Fifi's eight offspring to die in infancy. He died during an epidemic of sarcoptic mange in which two other infants also died, but several survived.

Genetic analysis using noninvasive DNA collection techniques is an important tool for exploring the outcomes of behavioural strategies employed by chimpanzees. Observation alone provides information about maternal relationships, but genetic analysis is necessary to determine paternity and paternal relationships. This information allows us to examine the success of male and female mating patterns, including possessiveness, opportunistic matings, consortships and inbreeding avoidance. Finally, our noninvasive approach should facilitate comparisons with other chimpanzee populations throughout East Africa, helping to define the relative genetic diversity and differentiation of the Gombe population overall, and hence its isolation from other chimpanzee populations.

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Appendix I

A = number of faeces/number of hair extractions used to determine genotype at each locus.
B = number of PCR reactions (faeces/hair)/number of separate days of PCR runs.
C = number of PCR reactions with allelic dropout using faeces/hair extraction.
D = Inconsistencies in amplification are coded with the following designations:
a = extra bands appearing along with the genotyped bands (these reactions are included in the tally in column B).
b = PCR reactions due to one DNA extraction consistently amplifying incorrectly and not included in column B or C.
c = incorrect bands appearing in one specific PCR run date only.
d = single PCR reaction yielding incorrect bands, not included in column B.
e = inconsistent bands are out of the expected allele size range.
In column D, individual PCR reactions with characteristics a–e are separated by commas. Numbers indicate multiple reactions with the same characteristics. Each case is the result of a faecal extraction unless noted with an H for hair.
– = no reactions and no extractions for faeces or hair used for an individual at this primer.
* = homozygotes.
*? = homozygotes with less than seven confirming PCR reactions.

AL = Atlas	FE = Ferdinand	GL = Gimble	SI = Schweini
AO = Apollo	FF = Fifi	GM = Gremlin	SL = Sheldon
AP = Aphro	FI = Fred	JF = Jiffy	SP = Sparrow
BE = Beethoven	FN = Fanni	JK = Jackson	TA = Tanga
CD = Candy	FO = Faustino	KS = Kris	TB = Tubi
CN = Conoco	FR = Frodo	MEL = Mel	TN = Titan
DB = Darbee	FU = Fudge	PF = Prof	TT = Tita
DL = Dilly	GA = Gaia	PI = Patti	TZ = Trezia
EV = Evered	GB = Goblin	PX = Pax	WL = Wilkie
FD = Freud	GD = Galahad	SA = Sandi	

	D4S243				**D10S676**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	–/1	–/7/4	–/0		1/2	5/4/4	*	
AO	–/1	–/5/2	–/0		3/1	7/7/4	3/3	
AP					1/–	2/–/1	2/–	
BE	1/2	3/21/5	2/1	b	5/3	13/16/17	10/12	a3,ace2,d
CD	1/–	4/–/1	0/–					
CN	1/–	4/–/1	0/–		2/–	5/–/1	1/–	
DB								
DL	–/1	–/4/1	–/0		–/1	–/4/1	–/0	
EV	–/2	–/5/4	–/2		–/2	–/3/1	–/0	cd2H
FD	1/1	3/3/1	0/2	ae3H	5/2	9/6/8	5/4	ac2H,aH
FE	1/–	4/–/1	0/–		4/–	12/–/4	7/–	ac3
FF	1/1	2/4/2	1/0	ae,b2	2/1	4/4/5	3/0	
FI	–/1	–/6/3	–/0		–/2	–/11/3	*	
FN	1/–	4/–/1	0/–		2/–	8/–/3	0/–	ac2
FO	3/1	5/2/3	2/0		3/1	8/6/4	*	dH,ae,d
FR	2/1	4/6/3	1/0		1/1	2/5/3	1/0	
FU	1/–	12/–/3	*		1/–	3/–/1	0/–	
GA	3/–	20/–/7	*	d,b7	1/–	4/–/1	0/–	d
GB	2/–	3/–/2	0/–		3/1	13/5/7	*	
GD	3/–	8/–/2	2/–	a,d	10/–	20/–/8	16/–	ac2,a2,d
GL	1/1	2/3/2	0/0		3/1	5/3/3	4/1	
GM	1/1	3/9/4	1/1	b,ae,de	5/1	7/7/7	7/0	
JF	2/–	13/–/5	10/–	a,d	2/–	3/–/2	1/–	
JK	2/–	13/–/4	0/–		2/–	4/–/1	0/–	
KS	1/1	9/1/3	4/1	a2	1/1	3/2/3	0/2	a2H,cd2H, a
MEL	2/–	4/–/2	4/–	a,d				
PF	4/–	9/–/1	0/–		4/–	9/–/1	1/–	
PI	1/1	6/2/4	0/0	a,de	1/1	2/3/3	2/1	
PX	1/–	3/–/1	*?		1/–	4/–/1	2/–	

Appendix I *Continued*

	D4S243 A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	**D10S676** A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
SA								
SI	3/–	7/–/3	0/–	a2	2/–	3/–/2	1/–	
SL	–/1	–/8/4	–/7		–/1	–/6/3	–/1	dH,d4,ac2
SP	1/–	4/–/1	0/–	a	1/–	2/–/1	0/–	
TA	1/1	3/2/2	3/0		2/1	2/2/3	0/0	
TB	3/–	8/–/3	0/–		2/–	1/–/4	1/–	
TN	2/–	7/–/3	4/–	ac3	1/–	6/–/2	3/–	ae,de
TT	1/–	3/–/1	0/–					
TZ	–/1	–/3/1	–/0					
WL	2/1	8/8/4	1/0	ae	3/1	5/6/4	0/0	
	D2S433 A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	**D18S536** A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	2/1	2/5/4	1/3	ace3H,ae2H	3/2	6/5/7	3/0	
AO	2/1	4/5/3	*	ae	3/1	11/4/5	*	
AP	1/–	2/–/1	0/–					
BE	1/2	5/8/3	*	aeH,ace3H,ae	6/3	13/9/10	10/1	a,d,de
CD								
CN					1/–	4/–/1	0/–	
DB	1/–	3/–/1	3/–					
DL					–/1	–/5/1	–/0	
EV	–/2	–/8/3	–/7	ace3H,aeH,deH	–/2	–/4/3	–/1	
FD	3/–	5/–/2	2/–		4/2	7/6/6	2/5	
FE	2/–	10/–/3	*	b	4/–	7/–/4	5/–	ac2
FF	1/1	4/5/3	1/0	ae2	–/1	–/3/3	–/0	dH
FI	–/1	–/12/3	–/1	ace7H,aeH	–/1	–/4/2	–/0	aeH
FN	2/–	5/–/1	1/–	ae	2/–	5/–/1	1/–	ae2
FO	2/–	3/–/1	0/–	ae	3/1	5/5/6	4/2	aH,d1,ac3,cd2
FR	5/1	6/5/3	0/0	ace5H	3/1	5/2/5	3/0	
FU	1/–	4/–/1	0/–	ae	1/–	4/–/1	0/–	
GA	1/–	4/–/1	0/–		1/–	6/–/2	0/–	b2
GB	3/1	7/2/3	0/2	acH,ac,ae	2/1	7/2/3	0/1	
GD	7/–	13/–/4	7/–	ae	8/–	24/–/7	*	a3,cd2,d,de
GL	2/1	9/2/4	*	aeH,ae	2/1	5/2/5	2/0	
GM	2/2	5/9/6	*	ace3H,aeH,dH,cd3	6/1	11/8/8	7/1	ac2,b,a2
JF	1/–	5/–/3	4/–	ae	1/–	4/–/1	0/–	
JK	2/–	3/–/2	0/–		2/–	8/–/2	*	
KS	2/–	10/–/3	*	ae2,d	1/–	6/–/2	*?	
MEL					2/–	2/–/1	1/–	
PF	5/–	10/–/1	*		5/–	9/–/3	2/–	
PI	1/–	7/–/3	2/–	ace2	1/1	7/3/4	1/1	ace2,ae,cd3
PX	1/–	3/–/1	0/–		1/–	3/–/1	0/–	
SA					2/1	5/2/3	0/2	
SI	1/–	3/–/1	0/–		2/–	7/–/4	6/–	
SL	1/1	1/1/2	1/1		–/1	–/5/2	–/1	aeH,dH
SP	1/–	4/–/2	0/–		1/–	5/–/3	0/–	
TA	1/1	3/4/2	0/2	ae3H	1/1	1/6/2	1/4	
TB	–/1	–/5/2	–/5		2/–	6/–/2	0/–	
TN	2/–	8/–/4	*		2/–	30/–/9	*	ae2,a4,d
TT	1/–	3/–/1	*?					
TZ	–/1	–/2/1	–/0					
WL	3/1	6/1/2	1/0		3/1	9/3/2	0/0	a

Appendix I *Continued*

	D19S431				**D4S1627**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	1/1	7/2/5	*		–/1	–/4/1	–/0	
AO	2/1	5/5/3	4/1	a	1/1	1/2/1	1/0	d
AP								
BE	3/2	6/17/7	*	cd2H,a2	1/1	1/4/2	1/1	
CD					1/–	3/–/1	*?	
CN	2/–	7/–/2	*	ae,d	1/–	4/–/1	0/–	ae2
DB	1/–	2/–/1	*?					
DL	–/1	–/3/1	*?		–/1	–/4/1	–/0	
EV	–/2	–/3/2	–/1	dH	–/1	–/3/2	–/1	
FD	3/1	8/1/5	2/1	ac2	1/1	3/1/2	1/0	
FE	1/–	2/–/1	0/–	cd4	1/–	2/–/1	0/–	
FF	2/1	4/5/3	*	ac4,a	–/1	–/4/1	–/0	
FI	–/1	–/6/2	–/0		–/1	–/3/1	–/0	
FN	2/–	4/–/2	2/–		3/–	9/–/2	4/–	a2
FO	2/1	5/3/4	1/0	cd2,deH	–/1	–/4/1	–/0	
FR	3/1	5/1/5	2/0		1/1	3/2/2	0	
FU	1/–	8/–/2	*		1/–	8/–/2	*	
GA	1/–	7/–/2	0/–		1/–	3/–/1	0/–	
GB	4/–	14/–/6	2/–	dH	2/–	10/–/3	*	
GD	4/–	15/–/6	4/–	ac6,a	3/–	5/–/2	0/–	
GL	1/1	6/8/4	*	aH,b	1/1	1/1/1	0/0	
GM	4/1	7/7/4	5/2	ac4H,ac2,dH,cd4	1/1	2/2/1	0/0	
JF	2/–	5/–/2	*?		1/–	3/–/2	0/–	ae
JK	2/–	4/–/1	0/–		2/–	6/–/2	0/–	
KS	1/–	9/–/3	*		1/–	5/–/2	*?	a
MEL					1/–	1/–/1	*?	
PF	2/–	4/–/1	0/–		3/–	8/–/2	0/–	
PI	1/1	2/5/3	2/3	ac2	1/1	6/2/3	3/1	aH
PX	1/–	5/–/2	2/–		1/–	6/–/2	0/–	d
SA	1/1	4/1/2	*?		1/1	2/1/2	2/1	
SI	2/–	5/–/2	4/–	a,d	3/–	8/–/3	6/–	a2
SL	–/1	–/5/3	–/2		1/1	1/2/3	*?	
SP	1/–	4/–/1	0/–		1/–	4/–/1	0/–	
TA	–/1	–/4/1	–/3		–/1	–/4/2	–/1	
TB	2/–	4/–/1	0		1/1	1/7/3	1/5	ae
TN	2/–	11/–/4	5/–	ac5,a,d	1/–	16/–/4	*	ac2
TT	1/–	4/–/1	1/–					
TZ	–/1	–/3/1	*?					
WL	4/1	11/3/3	0/0		1/1	2/2/1	0/0	

	D9S905				**D2S1326**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	1/1	2/4/2	1/0		–/1	–/5/2	–/0	
AO	–/1	–/5/2	–/0	(a,d)c2	–/1	–/5/2	–/0	
AP								
BE	5/2	10/7/9	8/1	a	–/2	–/8/1	–/2	
CD					1/–	4/–/1	2/–	
CN	2/–	5/–/1	3/–		1/–	4/–/1	0/–	
DB								
DL	–/1	–/4/1	–/0		–/1	–/4/1	–/0	
EV	–/2	–/3/2	–/1		–/1	–/4/2	–/2	

Appendix I *Continued*

	D9S905				**D2S1326**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
FD	3/2	5/5/4	3/4	dH	2/–	2/–/2	2/–	deH
FE	3/–	9/–/3	6/–	cd2,d	1/–	6/–/1	6/–	cde7,a
FF	2/1	6/5/4	4/1	cd4,d2	1/1	2/4/3	1/0	
FI	–/2	–/9/3	–/0		–/2	–/3/2	–/0	
FN	2/–	7/–/3	*		1/1	1/7/2	0/3	deH
FO	1/1	5/4/4	5/0	cd2H,a	1/1	7/2/2	0/0	
FR	1/1	1/4/3	0/0		1/1	3/2/3	1/0	
FU	1/–	6/–/3	0/–		1/–	8/–/2	4/–	
GA	1/–	2/–/2	0/–		1/–	8/–/2	1/–	
GB	3/1	9/2/3	2/0	a2	3/–	7/–/3	5/–	
GD	7/–	18/–/8	*	(d5,a)c,d2,a	1/–	9/–/3	*	
GL	2/1	5/4/4	5/1		1/–	4/–/1	0/–	
GM	2/2	7/5/5	4/0	(d7,a)c	–/1	–/4/2	–/1	
JF	2/–	8/–/2	*	a	1/–	4/–/2	*?	
JK	2/–	8/–/2	*		1/–	6/–/1	0/–	
KS	1/–	3/–/1	0/–		1/–	10/–/3	7/–	ae2
MEL	2/–	4/–/2	*?					
PF	3/–	7/–/1	*	ae	5/–	8/–/2	8/–	
PI	1/1	4/1/2	0/1	a,de	1/–	6/–/2	3/–	
PX	1/–	3/–/1	0/–		1/–	2/–/2	0/–	
SA	1/1	6/1/3	*					
SI	1/–	4/–/2	3/–	a	1/–	3/–/1	2/–	
SL	–/1	–/2/1	*?	de4	1/–	2/–/1	2/–	
SP	1/–	4/–/1	0/–		1/–	2/–/1	0/–	
TA	2/1	2/8/6	2/2	aeH,aH,de2	–/1	–/5/2	–/2	dH
TB	2/–	5/–/2	0/–		–/1	–/5/3	–/3	
TN	2/–	6/–/3	3/–	cde3	1/–	4/–/1	1/–	
TT	1/–	3/–/1	*?					
TZ								
WL	3/1	8/3/2	1/0	a	–/1	–/4/2	–/0	

	D20S470				**D2S1333**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	–/1	–/3/1	–/0		–/1	–/3/2	–/0	
AO	–/1	–/7/2	*		2/1	2/1/2	1/0	d
AP	1/–	7/–/2	4/–	ac3				
BE	–/1	–/6/2	–/2		1/2	2/6/2	0/0	
CD	1/–	2/–/2	0/–					
CN	1/–	10/–/3	*	b,ac2	1/–	4/–/1	0/–	
DB	1/–	4/–/2	*?					
DL	–/1	–/3/1	–/0		–/1	–/4/1	*?	
EV	–/1	–/4/2	–/2		–/1	–/6/2	–/2	
FD	2/–	11/–/3	5/–	ac2	1/–	3/–/1	0/–	
FE					1/–	4/–/2	*?	
FF	1/1	3/1/1	0/0	aH	1/1	2/1/2	2/0	
FI	–/1	–/4/1	–/0		–/1	–/3/2	–/0	
FN	1/1	3/4/2	*		2/–	3/–/1	1/–	
FO	–/1	–/7/2	*		1/–	3/–/1	0/–	
FR	2/1	5/2/3	*	a2	1/1	2/1/2	0/0	
FU	1/–	8/–/2	0/–		1/–	3/–/1	0/–	
GA	1/–	6/–/2	3/–	ac2	1/–	4/–/1	1/–	a

Appendix I *Continued*

	D20S470				**D2S1333**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
GB	1/1	1/5/2	1/2		1/–	3/–/1	0/–	
GD	2/–	4/–/2	1/–		2/–	4/–/1	0/–	
GL	1/1	4/3/2	*		1/1	2/4/3	2/0	a
GM	–/1	–/6/3	–/4		1/1	2/1/2	0/0	
JF	1/–	6/–/2	*?		2/–	6/–/3	*?	
JK	1/–	8/–/3	*		2/–	3/–/1	0/–	
KS	1/–	7/–/2	4/–		1/1	4/1/2	3/1	
MEL								
PF	2/–	5/–/2	1/–		3/–	7/–/2	*	
PI	1/–	4/–/1	1/–		1/–	3/–/1	0/–	
PX	1/–	3/–/1	0/–	d	1/–	3/–/1	0/–	
SA	1/1	5/2/2	4/1		1/1	5/1/3	*?	
SI	1/–	3/–/1	*?	a	2/–	4/–/2	*?	
SL	1/–	2/–/1	*?		–/1	–/4/3	–/4	
SP	1/–	3/–/1	2/–		1/–	2/–/1	0/–	
TA	–/1	–/3/1	–/2		–/1	–/2/1	–/1	d
TB	–/1	–/10/4	*		2/–	4/–/2	0/–	
TN	1/–	3/–/1	0/–		1/–	6/–/2	2/–	a,d
TT	1/–	3/–/2	0/–					
TZ	–/1	–/4/2	*?					
WL	–/1	–/12/4	*		1/–	4/–/2	0/–	

	D9S302				**D9S922**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	C extracts f/h	A pcr rxns f/h/days	B dropout f/h	D extra bands
AL	–/1	–/3/1	–/0		–/1	–/3/2	–/0	
AO	–/1	–/4/1	*?		–/1	–/5/2	*?	
AP								
BE	–/1	–/5/2	–/2		1/2	1/6/2	1/0	
CD					1/–	1/–/1	0/–	
CN	1/–	8/–/2	5/–	a	1/–	4/–/1	2/–	
DB	1/–	1/–/1	0/–					
DL	–/1	–/3/1	–/0		–/1	–/4/1	–/0	
EV	–/1	–/3/1	–/0		–/1	–/3/2	–/1	
FD	2/–	5/–/1	3/–	a	1/1	3/1/2	1/1	
FE					1/–	1/–/1	0/–	b
FF	2/–	6/–/2	2/–		1/1	3/3/4	0/0	
FI	–/1	–/4/1	–/0		–/2	–/4/2	–/0	
FN	1/1	1/3/1	*?		–/1	–/2/1	–/0	
FO	–/1	–/3/1	–/0		1/–	2/–/1	0/–	
FR	1/1	2/2/1	0/0		1/1	1/1/1	0/0	
FU	1/–	4/–/1	0/–		1/–	3/–/1	0/–	
GA	1/–	3/–/1	0/–		1/–	8/–/1	0/–	
GB	1/1	1/2/1	1/0		1/–	2/–/2	1/–	
GD	2/–	7/–/2	*		3/–	3/–/3	1/–	
GL	1/1	3/1/1	*?		1/–	4/–/1	*?	
GM	–/1	–/7/2	*		1/1	2/6/2	1/0	
JF	1/–	2/–/1	*?		1/–	7/–/3	5/–	
JK	1/–	4/–/1	0/–		1/–	2/–/1	0/–	
KS	1/–	4/–/1	*?		1/–	2/–/1	0/–	
MEL					1/–	1/–/1	?	
PF	2/–	4/–/2	*?		4/–	9/–/1	0/–	

Appendix I *Continued*

	D9S302				**D9S922**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	C extracts f/h	A pcr rxns f/h/days	B dropout f/h	D extra bands
PI	1/–	3/–/1	1/–		1/–	2/–/1	1/–	
PX	1/–	4/–/2	*?					
SA	1/1	2/1/2	*?					
SI	1/–	3/–/1	1/–		1/–	1/–/1	*?	
SL	1/1	2/1/2	2/0	a	–/1	–/2/2	–/2	
SP	1/–	3/–/1	0/–		1/–	4/–/1	0/–	
TA	–/1	–/3/1	*?		–/1	–/3/1	*?	
TB	–/1	–/6/3	–/5	a	2/1	2/6/4	0/3	cd2H
TN	1/–	3/–/1	0/–		1/–	3/–/2	2/–	
TT	1/–	4/–/1	0/–					
TZ	–/1	–/4/1	–/2					
WL	–/1	–/4/1	–/0		1/1	2/7/3	1/0	a

	D1S158				**D11S1366**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	–/1	–/2/1	–/0		–/1	–/6/3	–/0	
AO	–/1	–/3/1	–/0		–/1	–/7/3	–/0	dH
AP					1/–	3/–/1	3/–	
BE	–/2	–/7/2	*		–/2	–/13/2	–/6	(aH,dH)c
CD					1/–	2/–/1	1/–	
CN	1/–	4/–/1	0/–		2/–	4/–/1	4/–	
DB					1/–	1/–/1	?	
DL	–/1	–/4/1	–/0		–/1	–/4/1	–/0	
EV	–/1	–/9/3	–/5	a	–/1	–/6/2	–/5	
FD	1/1	1/1/1	0/1		2/1	4/4/4	2/4	dH,cde2H,de
FE	1/–	11/–/2	*		1/–	12/–/2	11/–	ac3,a
FF	1/1	1/1/1	0/0	(de,deH2)c	1/1	1/2/2	1/0	
FI	–/1	–/2/1	–/0	cde4H	–/2	–/2/1	–/0	
FN	–/1	–/2/1	*?		1/–	1/–/1	0/–	
FO	1/–	1/–/1	0/–		1/–	3/–/1	2/–	
FR	1/1	1/1/1	0/0		1/1	1/1/1	0/0	
FU	1/–	2/–/1	0/–		1/–	3/–/1	*?	
GA	3/–	3/–/1	1/–		1/–	9/–/3	3/–	a,d,(a,d)c
GB	3/–	10/–/2	*		1/–	2/–/1	*?	
GD	3/–	6/–/2	3/–		3/–	6/–/2	4/–	
GL	1/–	2/–/1	0/–		1/–	4/–/1	0/–	
GM	–/1	–/3/1	–/1		–/1	–/3/1	–/0	
JF	1/–	2/–/1	*?		1/–	1/–/1	?	
JK	2/–	4/–/1	*?		1/–	3/–/1	1/–	
KS	1/–	10/–/2	*	de	1/–	7/–/2	7/–	
MEL	2/–	3/–/2	1/–	a				
PF	4/–	7/–/1	0/–		2/–	3/–/1	*?	
PI	1/–	4/–/1	*?		1/–	4/–/1	4/–	
PX					1/–	1/–/1	?	
SA	1/–	2/–/1	0/–		–/1	–/3/1	–/0	
SI	1/–	8/–/2	*		1/–	4/–/2	4/–	a
SL	–/1	–/3/2	–/2		1/–	1/–/1	?	
SP	1/–	4/–/1	*?		1/–	4/–/1	0/–	
TA	–/1	–/1/1	–/0		–/1	–/7/2	–/4	
TB	–/1	–/10/2	*	a2	–/1	–/2/1	–/1	dH

Appendix I *Continued*

	D1S158				**D11S1366**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
TN	1/–	4/–/1	2/–		1/–	3/–/1	1/–	
TT					1/–	3/–/1	1/–	
TZ	–/1	–/1/1	?		–/1	–/3/1	–/0	
WL	1/1	2/1/1	0/0		–/1	–/3/1	–/0	
	D18S851				**HUMFABP**			
	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands	A extracts f/h	B pcr rxns f/h/days	C dropout f/h	D extra bands
AL	–/1	–/3/1	–/0		–/1	–/12/3	*	
AO	1/1	1/3/1	1/0		–/1	–/5/2	–/2	
AP	1/–	4/–/1	2/–					
BE	–/1	–/7/2	*		1/2	1/18/3	1/5	
CD	1/–	1/–/1	?		1/–	4/–/1	*?	
CN	1/–	7/–/2	*		2/–	6/–/1	*?	
DB	1/–	1/–/1	?					
DL	–/1	–/3/1	*?		–/1	–/4/1	–/0	
EV	–/1	–/3/1	–/0		–/1	–/4/1	–/2	
FD	1/–	7/–/2	*		1/1	3/1/3	*	aeH
FE					2/–	8/–/3	*	ae
FF	1/1	2/1/1	*?		–/1	–/1/1	–/0	
FI	–/1	–/4/1	*?		–/1	–/2/1	–/0	
FN	1/1	1/3/1	*?		2/–	6/–/2	*	a3
FO	–/1	–/3/1	–/0		1/–	2/–/1	*	
FR	1/1	2/2/1	*?		1/1	3/1/2	*	
FU	1/–	3/–/1	*?		1/–	4/–/2	*	
GA	1/–	3/–/1	0/–		3/–	3/–/1	1/–	
GB	1/1	1/2/1	1/0	a				
GD	2/–	4/–/2	0/–		3/–	8/–/2	3/–	ae
GL	1/1	3/1/1	0/1					
GM	–/1	–/3/1	–/0		1/1	1/5/3	*	
JF	1/–	2/–/1	0/–		2/–	5/–/3	4/–	ae2,de
JK	1/–	4/–/1	0/–		2/–	12/–/4	*	ae2,de
KS	1/–	4/–/1	1/–		1/–	9/–/3	7/–	ae2,de
MEL								
PF	2/–	4/–/1	2/–		4/–	9/–/1	*	
PI	1/–	3/–/1	0/–		1/–	3/–/1	1/–	
PX	1/–	6/–/2	2/–					
SA	1/1	4/1/2	*?					
SI	1/–	3/–/1	0/–		1/–	5/–/2	*?	
SL	1/–	1/–/1	?		–/1	–/3/1	*?	
SP	1/–	3/–/1	0/–					
TA	1/1	3/2/2	*?		1/1	2/1/1	0/0	
TB	–/1	–/7/2	–/3		1/1	2/2/3	*	aeH
TN	1/–	3/–/1	*?		1/–	4/–/2	*?	
TT	1/–	7/–/2	2/–					
TZ	–/2	–/3/2	–/0					
WL	–/1	–/4/1	–/0		1/–	1/–/1	*	

Appendix II

Explanation of discrepancies between our study and the results of Morin *et al.* (1994) regarding exclusions of our designated paternal male in their study. Morin *et al.*'s alleles are presented below

	Mfd3		Mfd18		Mfd23		Mfd32		FABP		Pla2a		Rena4		LL	
I																
Fifi (mother)	5	9	6	10	1	1	5	9	4	7	7	7 *	3	3	12	12 *
Fanni (offspring)	9	8	1	10	1	22	5	5	4	4	6	6 *	3	3	11	4
Goblin (father)	5	9	1	4	4	22	6	5	4	7	7	6	3	3	4	4
II																
Gremlin (offspring)	5	5	1	10	24	24	9	6	6	6	7	7	2	2 *	8	4
Evered (father)	5	9	1	4	24	14	9	6	4	4(6) *	7	7	3	3 *	7	4
III																
Dominie (mother)	5	9	4	10	1	26	9	9	5	5 *	7	9	3	3	3	3*
Dilly (offspring)	5	5	6	10	26	20	9	9	5	6	9	9	3	3	4	3
Beethoven (father)	5	5					9	5	3(5)	3(7)	9	9	3	3	13	13 *

Underlined alleles indicate discrepancies. Genotypes with a * indicate homozygosity which may be due to allelic dropout.
Numbers in parentheses are the allele values from our study, Table 1. FABP is the only locus that both studies included.
I: Mfd3, mismatch may be stutter, Fanni has the only eight in the population; Pla2a, not a mismatch for Fanni/Goblin, but Fanni/Fifi is a mismatch; LL, not a mismatch for Fanni/Goblin, but Fanni/Fifi is a mismatch.
II: FABP, Evered incorrectly typed as homozygote, accounting for mismatch (4,6 is the correct genotype); Rena4, both Gremlin and Evered homozygotes, and dropout alleles may not have been discovered.
III: FABP, Beethoven incorrectly scored at this locus. (5,7 is the correct genotype) [Morin (personal communication) agreed that Beethoven's DNA did not amplify well], if Dominie is falsely homozygotic (i.e. 5,6), then Beethoven would not be excluded; LL, both Dominie and Beethoven may be falsely homozygotic.