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Paternity assessment in wild groups of toque macaques *Macaca sinica* at Polonnaruwa, Sri Lanka using molecular markers

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Abstract

Genetic variation at four microsatellite loci in conjunction with that at a highly variable allozyme locus was used to analyse paternity over a 12-year period in 13 social groups of toque macaques *Macaca sinica* inhabiting a natural forest in Polonnaruwa, Sri Lanka. Paternity exclusion analysis revealed that the set of offspring produced by a female usually consists of half-siblings because few males father more than one offspring with a particular female. No evidence of offspring produced by matings between first degree relatives was found. The social unit in toque macaques was not identical to the reproductive unit and the possibility of paternity by males outside the social group should be considered when estimating male reproductive output. Although it was common for multiple males to father offspring in a social group each year, reproduction within a group during a breeding season tended to be limited to a few males. The mean number of males reproducing per group per year was independent of the number of males in a group. The paternity data suggests that many males may father relatively few offspring during their entire lives and that the effective population size for toque macaques may be much smaller than indicated by demographic data.

Keywords: microsatellites, paternity, reproductive output, toque macaques

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Introduction

The social behaviour of terrestrial and semiterrestrial cercopithicines, particularly baboons, macaques and vervets, has been more intensively studied than any other group of nonhominoid primates (Melnick & Pearl 1987). Furthermore, the understanding of social behaviour in these species has to a large extent influenced perceptions of the structure and function of social interactions in primates in general. Yet despite numerous long-term studies (Dittus 1977a, 1988; Packer 1979; van Noordwijk & van Schaik 1987; Altmann *et al*. 1988; Cheney *et al*. 1988), theories concerning the evolution and adaptiveness of some aspects of social behaviour in these species have proven difficult to evaluate because of the difficulty in reliably determining paternity in free-ranging populations of primates. It is rarely possible to be certain that all copulations with a particular oestrus female have been observed and females may mate with multiple males. Until recently, it has only been possible to infer male reproductive output from rates of sexual activity. Unfortunately, rates of sexual activity are not necessarily good predictors of the number of offspring sired (Stern & Smith 1984; Shively & Smith 1985) and imply that behavioural observations of reproductive activity cannot be used to assess paternity accurately and male patterns of reproduction in natural populations.

However, by using novel genetic markers it is possible to obtain statistically acceptable probabilities of paternity in free-ranging primates even when the pool of potential

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fathers is uncertain or incompletely sampled (Hayasaka *et al*. 1986; Morin & Woodruff 1992). Traditional allozyme markers have been of only limited use for resolving paternity in primates because variation at the underlying loci is generally too low (Nozawa *et al*. 1982; Dracopoli *et al*. 1983; Shotake & Nozawa 1984; Morin & Woodruff 1992). Recently, PCR amplification of microsatellites has proven to be a viable alternative approach for analysing genetically variable loci in primates (Inoue & Takenaka 1993; Morin *et al*. 1993). The high degree of polymorphism at many microsatellite loci coupled with the fact that the alleles are codominant, effectively neutral and inherited in a Mendelian fashion makes them particularly good markers for paternity analysis (Queller *et al*. 1993).

This study combines long-term demographic and behavioural data (Dittus 1977a, 1979, 1988) with a genetic analysis of paternity in order to examine some patterns of male reproduction in a natural population of toque macaques *Macaca sinica* living in Polonnaruwa, Sri Lanka. Although groups containing a single male with multiple females are not uncommon, toque macaques typically live in multimale, multifemale groups and have a promiscuous mating system. We have applied paternity exclusion analysis to offspring born in 13 social groups over a 12-year period. This investigation is the first to couple long-term demographic data from a large number of social groups in a single population with a genetic analysis of paternity in order to examine male patterns of reproduction in cercopithicine primates.

Materials and methods

Study population

Demographic and behavioural data, as well as blood samples for genetic analysis were collected from a population of toque macaques located in the Nature Sanctuary and Archaeological Reserve at Polonnaruwa, Sri Lanka. This population has been under continuous study since 1968, and in 1987 comprised over 600 individuals in 23 social groups. The natural evergreen forest inhabited by the macaques, and many aspects of their demography, ecology and behaviour, as well as the methods of study, have been described previously (Dittus 1977a,b, 1979, 1988). All macaques in this population were individually identifiable by their natural markings (Dittus & Thorington 1981) and were habituated to the presence of human observers. Females bear a single offspring about once in 18 months (Dittus 1975). Twin births are extremely rare and none was involved in this study. The birthdates and ages of most animals born after 1968 are known. The ages of those born earlier, or of those immigrating into the study population, were estimated on the basis of their morphological development (Dittus 1988). Life histories were known for most individuals and matrilineal relationships were based on birth records.

Genetic analysis

The genetic data used to assess paternity were obtained from blood samples collected during 1986–87 from all members of 13 social groups within the study population. Methods of live-trapping and blood collection, preparation and storage are described elsewhere (Melnick *et al*. 1984; Hoelzer *et al*. 1994).

Several allozymes were assayed for phenotypic variation using standard starch and polyacrylamide gel electrophoresis techniques of which only the transferrin locus was found to contain sufficient polymorphism so as to be useful for paternity analysis. Previous mating experiments have shown the phenotypic variation for transferrin to be the products of codominant alleles at a single autosomal locus (Goodman & Wolf 1963).

Microsatellite length polymorphisms in toque macaques were surveyed using heterologous PCR primer pairs which amplify target CA repeats. Two sets of PCR primers (Mfd 125 and Mfd 23) designed from DNA sequences of humans (Weber *et al*. 1990; Morin & Woodruff 1992) and two sets (MFGT2 and MFGT17) designed from DNA sequences of Japanese macaques (Inoue & Takenaka 1993) amplified sufficiently polymorphic DNA products in toque macaques to be useful for paternity analysis.

Genomic DNA was extracted from whole blood as described elsewhere (Keane *et al*. 1994) and stored in 1/10 TE (1 mM Tris pH 7.5, 0.1 mM EDTA). One primer strand per set (10 pmol) was 5′ end-labelled with [γ-^{32}P] ATP by T4 polynucleotide kinase in a total reaction volume of 25 μL for 60 min at 37 °C. The entire 25-μL kinase reaction was added to sufficient buffer mix for 20–40 polymerase chain reactions without removing the unincorporated nucleotides. All polymerase chain reactions were carried out in 25-μL reactions containing 50–100 ng of DNA template, 10 mM Tris (pH 8.3), 50 mM KCl, 10 pmol of each oligonucleotide primer, 0.25–0.5 pmol of end-labelled primer, and 0.25 U *Taq* DNA polymerase. In addition, the reaction mix for Mfd 125 contained 2.5 mM $MgCl_2$ and 250 μM dNTPs and that for Mfd 23 contained 2.75 mM $MgCl_2$ and 250 μM dNTPs. For both Mfd 125 and Mfd 23, denaturation for 2 min at 94 °C was followed by amplification for 33 cycles of 1 min denaturation at 94 °C, 1.5 min annealing at 53 °C and 2.5 min extension at 72 °C with a final extension of 7 min at 72 °C. For MFGT2 the reaction mix also contained 7.5 mM $MgCl_2$ and 400 μM dNTPs and for MFGT17 the reaction mix also contained 7.0 mM $MgCl_2$ and 400 μM dNTPs. For both of these primer sets

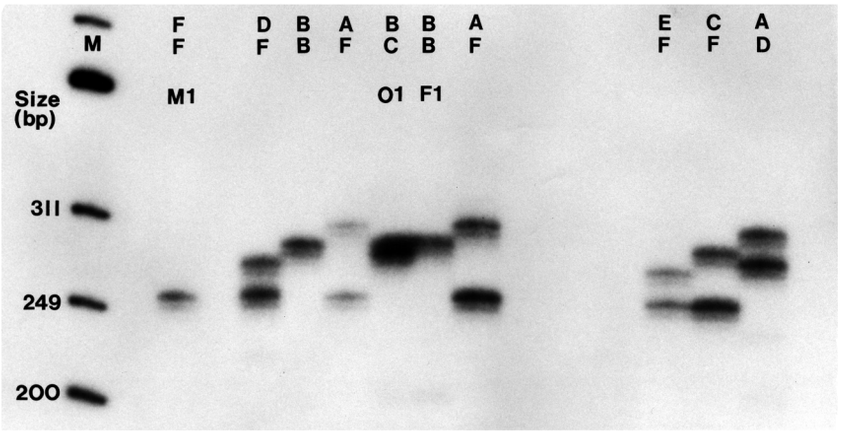

Fig. 1 Dinucleotide $(CA)_n$ repeat polymorphism detected by PCR with the primer pairs Mfd125 following denaturation polyacrylamide gel electrophoresis. PCR products were visualized by end-labelling one primer strand per set with [γ–^{32}P] ATP and autoradiographing gels. Individual genotypes are listed above the bands. The lane marked O1 contains DNA from an offspring of the female in the lane marked F1. The resident male in the lane marked M1 was excluded from paternity for this offspring because he did not posses the offspring's paternally inherited allele (C) at this locus. Lanes marked M contain *Hin*fI digested ΦX174 DNA with the fragment sizes indicated in base pairs.

denaturation for 3 min at 92 °C was followed by amplification for 28 cycles of 1 min denaturation at 92 °C, 2 min annealing at 55 °C and 3 min extension at 72 °C with a final extension time of 7 min at 72 °C. Reaction products (6 μL) were mixed with 4 μL of formamide stop solution (United States Biochemical Corp.), denatured at 95 °C for 3 min, snap-cooled in a water–ice bath and electrophoresed on 6% polyacrylamide, 50% urea sequencing gels at 1100 V for 2 h with Tris-borate buffer (0.089 M Tris, 0.089 M boric acid, and 2 mM EDTA). End-labelled *Hin*fI digested ΦX174 DNA was also run on all gels as a size marker. After electrophoresis, gels were transferred to exposed X-ray film, covered in plastic wrap and autoradiographed for 1–3 days at –80 °C without intensifying screens.

A total of 268 individuals from 13 social groups were typed for their phenotype at the transferrin locus and at the four microsatellite loci and electrophoretic phenotypes were interpreted as genotypes. Individual genotypes were identified on the basis of the relative migration of proteins/DNA fragments to each other and to the size standards on each gel (Fig. 1). Comparisons between microsatellite gels were made by the inclusion of amplified products run on previous gels. Genotypes were used to calculate allele frequencies by simple gene counting and form the basis for the paternity analysis.

Paternity analysis

In order to determine how effective the genetic markers we used were for determining the fathers of offspring born in this population to mothers whose genotypes were known, we calculated the probability that a male other than the actual father could possess by chance the paternally inherited alleles at the five loci typed. This probability is proportional to the number and frequency of alleles at each locus used for paternity analysis. Because alleles at all five loci used to type the individuals in this population are codominant, where all genotypes are detectable, we used the method of Selvin (1980) to calculate the probability of a randomly chosen male who is not the father possessing by chance all the paternal alleles in an offspring. Assuming Hardy–Weinberg equilibrium, this probability of nonexclusion (P_i), for the *i*-th locus, is given by the equation:

$$P_i = 1 - \sum_{j=1}^{n} \{p_j [1 - p_j]^2 + \sum\sum_{j>k} p_j p_k \{[1 - p_j]^3 + [1 - p_k]^3 + [p_j + p_k][1 - (p_j + p_k)]^2\}$$

where p_j and p_k are the frequencies of the *j*-th and *k*-th alleles and *n* is the number of alleles at the locus. When *m* loci are used for paternity analysis, the probability of a male other than the actual father possessing all the paternal alleles is given by:

$$P = \prod_{i=1}^{m} (P_i).$$

If the assumption of Hardy–Weinberg equilibrium is violated, then this method will overestimate the probability of excluding males that are not the father.

For each locus used in the paternity exclusion analysis, departures of observed genotype frequencies from Hardy–Weinberg expectations were tested using a *G*-test for goodness-of-fit. At each locus, adjacent genotype categories were combined when necessary to ensure that no genotype category had an expected value less than one and that less than 20% of the genotype categories had expected values less than five (Daniel 1987).

All sampled adult and subadult males present in an infant's natal group at the time of conception were considered to be possible fathers and were screened for paternity. In cases where all resident males were excluded from paternity, additional males from neighbouring groups were also screened for paternity. Male toque macaques are

not fully grown until 9–12 years of age (Cheverud *et al.* 1992), but all males that were at least 6 years of age at the time of an infant's conception were considered as potential fathers. Likely paternity for a particular infant was assigned to a male only if he possessed all five of the offspring's paternally inherited alleles.

A male was considered to have had the opportunity to father an offspring if he was resident in an offspring's natal group at the time the offspring was conceived. For all putative fathers we determined their proportion of an offspring fathered per opportunity by dividing the number of offspring they fathered by the number of conceptions that occurred while they were resident in a group. A male's proportion of an offspring fathered per opportunity when in a group that contains a given number of adult males was determined by dividing the number of offspring fathered by a male when in a group that contains x adult males by the number of conceptions that occurred when they were resident in a group that contained x adult males.

Results

Allele frequencies at each of the five loci are shown in Table 1. Genotype frequencies were not significantly different from those expected by Hardy–Weinberg equilibria for all but the Mfd23 locus (Table 2). Deviations from Hardy–Weinberg expectations at this locus means the cumulative probability of nonexclusion (P) of a male that is not the actual father from paternity calculated over all five loci will be an underestimation. Because the cumulative probability of nonexclusion for the four other loci for which the assumption of Hardy–Weinberg is not violated is 0.031, the actual probability of nonexclusion for all five loci will be between $P > 0.03$ and $P > 0.02$ (Table 2). Therefore, there is only a 2–3% chance of a random male that is not the actual father of an offspring possessing all the offspring's paternally inherited alleles and being misidentified as the offspring's father. However, if the loci are genetically linked or the pool of putative fathers for an offspring includes relatives of the actual father, the resolving power of the loci will be lower.

All possible pairwise G-tests ($n = 10$) of the observed distribution of individuals heterozygous at zero, one or both of the tested loci with the expected numbers were nonsignificant, suggesting that the alleles at the loci used for paternity analysis were independently inherited. Because there was a significant deviation from Hardy–Weinberg expectations at one locus, the observed proportions of heterozygotes at each locus were used to calculate the expected values. If the loci were linked, the observed number of individuals heterozygous at just one of the pair of loci should have been less than that expected by chance (Westneat 1987).

The probability of nonexclusion of an individual from paternity depends on his genetic relationship to the real father (and mother) and will be higher for a relative of the actual father than for an individual unrelated to the actual father. When considering a full-sibling of the true father, the probability of nonexclusion is less than 0.07 for the loci used in this study (Salmon & Brocteur 1978). Of the 56 offspring born in multimale groups for which we could assign likely paternity to a male that was resident in the offspring's natal group at the time of its conception (see below), there were only two cases (4%) where more than one resident male possessed all five paternally inherited alleles. This suggests that the proportion of offspring for which relatives of the true father may have been misidentified as fathers was small.

Table 2 Observed heterozygosity (H_o), expected heterozygosity (H_e) with random mating, G-test of goodness-of-fit to expected Hardy–Weinberg proportions, probability (P_i) of nonexclusion of a male that is not the actual father for each locus, and the cumulative probability (P) of nonexclusion for combined loci. * $P < 0.05$

Locus	H_o	H_e	G	P_i	P
Transferrin	0.638	0.640	6.60 (d.f. = 8)	0.60	0.60
MFGT2	0.879	0.815	28.16 (d.f. = 18)	0.37	0.22
MFGT17	0.819	0.811	30.68 (d.f. = 22)	0.37	0.08
Mfd125	0.767	0.766	17.58 (d.f. = 16)	0.38	0.03
Mfd23	0.581	0.515	*12.06 (d.f. = 5)	0.75	0.02

Table 1 Distribution of alleles at the transferrin, Mfd23, Mfd125, MFGT2 and MFGT17 loci in 13 troops of wild toque macaques

	Allele frequency									
Locus	A	B	C	D	E	F	G	H	I	J
Transferrin	0.034	0.239	0.229	0.500	–	–	–	–	–	–
Mfd23	0.047	0.335	0.609	0.009	–	–	–	–	–	–
Mfd125	0.055	0.295	0.118	0.344	0.030	0.096	0.049	0.006	0.008	–
MFGT2	0.060	0.165	0.152	0.231	0.145	0.241	0.006	–	–	–
MFGT17	0.064	0.071	0.117	0.055	0.286	0.261	0.120	0.008	0.002	0.017

Table 3 Example of the paternity exclusion analysis for two offspring born in group J during 1986. Allele designations at the five loci used in paternity analysis are shown for two offspring, their mothers and the only adult resident male in the group. The resident male (201) was the likely father of offspring 209 because he possessed all five of the paternally inherited alleles but was excluded from paternity for offspring 208 because he did not possess the paternally inherited alleles at the Mfd.125, MFGT-17 and MFGT-2 loci

	Locus				
Individual	Mfd.125	Mfd.23	MFGT-17	MFGT-2	TRANS
Female 203	BF	BD	AC	BG	CD
Offspring 208	FF	DD	CD	AB	DD
Female 204	CF	DD	AE	AC	CD
Offspring 209	CF	DD	EF	CF	DD
Male 201	CD	DD	FG	FG	DG

Between 1975 and 1986, there were 145 offspring born in our study population for which DNA samples were available from an offspring, its mother and at least one of the adult males resident in the offspring's natal troop at the time of its conception. Five of these offspring had a genotype that was inconsistent with that of their mother. Three of these mother–offspring dyads had an inconsistency at a single microsatellite locus, one had an inconsistency at the transferrin locus and one had an inconsistency at both a microsatellite locus and the transferrin locus. Because mutation rates at allozyme loci have generally been estimated to be on the order of 10^{-5}–10^{-6} per locus per generation (Hartl & Clark 1989), the latter two cases suggest that the DNA samples tested did not come from a true mother–offspring pair or that mutation rates are higher than generally estimated. Mutation rates at microsatellite loci have been estimated to be 10^{-2}–10^{-5} per locus per generation (Weber & Wong 1993) so finding three mother–offspring dyads with inconsistencies at a single microsatellite locus is compatible with the estimated mutation rate at microsatellite loci.

Considering only the 140 offspring whose genotypes were consistent with that of their mother, we were able to assign likely paternity to a single male for 77 of these offspring on the basis of the paternally inherited alleles

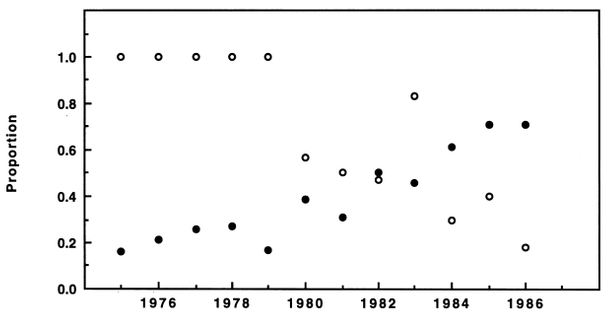

Fig. 2 The proportion of resident males in the study troops who could be genotyped each year (●) and the proportion of offspring born in the study groups each year for which paternity could not be assigned (○).

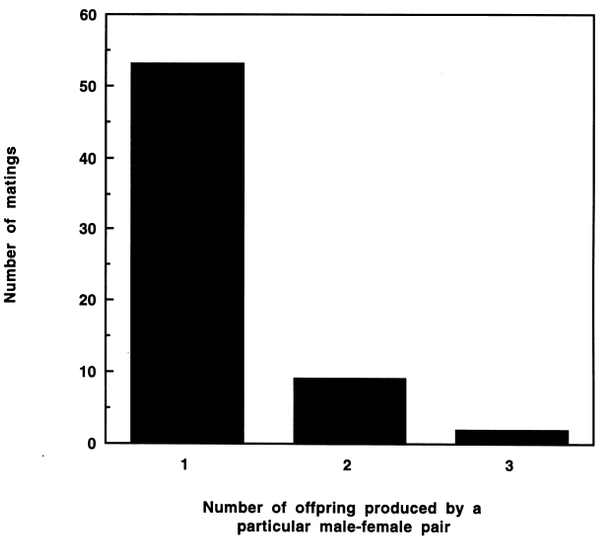

Fig. 3 The number of offspring produced by a particular male–female pair between 1975 and 1986. All females gave birth to just one offspring per pregnancy.

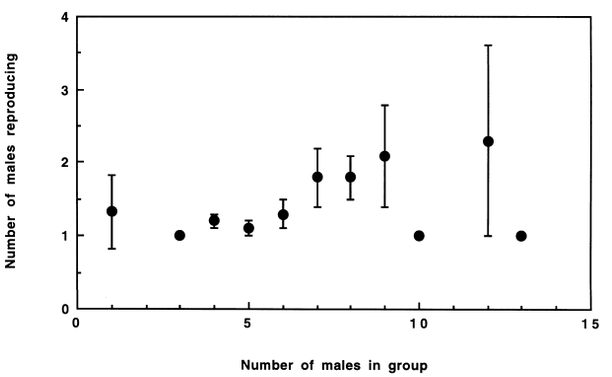

Fig. 4 The mean (± SE) number of males per group per year that fathered offspring between 1975 and 1986 as a function of the number of males in the group ($r = 0.41$, $P = 0.19$). The mean number of males reproducing in one-male groups was greater than one because some offspring in these groups were fathered by non-resident males.

Table 4 Paternity exclusions for 140 toque macaque offspring born between 1975 and 1986. All sampled adult males resident in the offspring's natal group at the time it was conceived were considered to be possible fathers. Males in bold face type could not be excluded from paternity. For the six underlined offspring, likely paternity was assigned to a nonresident male

Mother	Offspring	Resident adult males									
102	107	**101**									
102	109	**101**									
102	115	101	1025								
103	110	**101**									
106	105	101									
106	108	**101**									
106	113	101									
115	104	**101**									
115	111	**101**									
115	112	101									
202	206	**201**									
203	208	201									
204	209	**201**									
205	207	**201**									
501	502	**503**									
501	517	**503**									
501	522	**503**									
504	505	**503**									
504	514	503									
504	523	503									
506	513	**503**									
506	520	**503**									
509	515	**503**									
509	518	**503**									
510	511	**503**									
510	516	503									
524	507	503									
524	512	**503**									
524	525	**503**									
605	606	**601**	602	711	720	801	818	821	831	832	
605	618	602	711	801	818	821					
607	608	601	**602**	711	720	801	818	821	831	832	
609	610	601	602	711	720	801	818	821	831	832	**951**
611	612	601	**602**	711	720	801	818	821	831	832	
613	614	601	**602**	711	801	818	821	831			
616	604	601	602	711	801	818	**821**	831			
616	605	101									
616	607	101	1025								
616	620	**602**	711	801	**818**	**821**					
617	609	101	711	801	**818**						
617	721	801	**818**								
705	706	**701**	716	1025							
705	722	821	1025								
707	708	701	716	**820**							
707	715	1025									
707	1021	1025									
709	712	1025									
709	718	952	955	**958**							
710	717	821	1025								
710	832	1025									
712	713	701	716	719	820						
712	714	701	716	**818**	1025						
804	725	602	711	801	818	821					
804	802	601	602	711	720	801	818	821	831	832	
804	806	601	**602**	711	801	**818**	**821**	**831**			
807	805	**601**	602	711	720	801	818	821	831	832	
807	809	601	602	711	801	818	821	**831**			
807	829	602	711	801	818						

Table 4 *Continued*

Mother	Offspring	Resident adult males									
807	833	101	711	801	818						
811	808	601	602	711	801	818	821	831	**952**		
811	812	601	602	711	720	801	**818**	821	831	832	
811	815	601	602	711	801	818	821	831			
811	816	101	711	818							
814	726	711	801	818							
814	813	601	602	**711**	720	801	818	821	831	832	
814	822	101									
814	835	601	**602**	711	801	818	821	831			
816	817	601	602	**711**	801	818	821	831			
816	819	601	602	**711**	801	818	821	831			
822	724	711	801	**818**							
822	824	601	602	711	801	**818**	821	831			
826	803	601	602	**711**	801	818	821	831			
826	804	101	711	818							
826	825	601	602	711	801	818	821	831	901	902	918
830	827	601	602	711	**801**	818	821	831			
830	828	601	602	711	801	818	821	831			
833	810	601	602	711	801	818	821	831			
904	915	901	**902**								
905	908	703	901	902	917	918					
905	913	703	901	902	918						
906	911	703	901	902	917	918					
909	910	703	901	902	917	918					
916	912	703	901	902	917	918					
916	914	703	901	**902**	918						
929	930	926									
929	939	925									
932	933	926									
942	944	**941**									
953	954	**951**	952	955	958	969	970	972			
956	957	951	952	**955**	958	969	970	972			
956	967	952	958								
959	960	951	952	955	958						
959	966	952	958								
961	962	951	952	955	958						
961	964	952	958								
980	977	975									
981	979	**975**									
982	978	975									
990	719	701	702	820	1011						
990	991	702	**985**	986	988						
992	993	**985**	986	988							
994	989	701	702	952	**958**	1011					
994	997	701	**702**	831							
994	1018	701	820	1011							
999	723	701	702	**820**	1011						
999	996	701	702	831							
999	1000	701	702	1011							
999	1001	701	820	1012							
1001	704	701	702	831							
1001	1002	702	**985**	986	988						
1003	1004	**985**	986	988							
1013	1029	**602**	1010	1012	1014						
1013	1035	602	1010	1012	1014						
1013	1040	601	**1010**	1011	1012	1014					
1013	1042	602	701	820	1012						
1013	1052	**1010**	1011	1012	1014						

Table 4 *Continued*

Mother	Offspring	Resident adult males							
015	1024	602	1010	1012	1014				
1015	1038	601	1010	1011	1012	1014			
1015	1041	702	**955**	1010	1011	1012	1014	1025	1049
1015	1054	1010	1011	**1012**	1014				
1019	1020	702	1010	**1011**	1012	1014	1025	1049	
1019	1032	601	986	**1010**	1011	1012	1014		
1022	1023	702	**820**	1010	1011	1012	1014	1025	1049
1027	1022	602	702	1010	**1011**	1012	1014		
1027	1028	702	1010	1011	**1012**	1014	1025	1049	
1027	1036	602	**1010**	1012	1014				
1027	1037	**1010**	1011	1012	1014				
1027	1053	601	986	**1010**	1011	1012	1014		
1030	1016	**602**	1010	1012	1014				
1030	1031	702	1010	1011	1012	**1014**	1025	1049	
1030	1046	1010	1011	1012	1014				
1030	1047	701	820						
1030	1051	602	1010	1012	1014				
1033	1026	601	986	1010	1011	1012	1014		
1033	1034	702	1010	1011	1012	1014	1025	1049	
1033	1050	602	1010	1012	1014				
1042	1044	**1010**	1011	1012	1014	1025	1049		
1042	1045	601	**986**	1010	1011	1012	1014		
1047	1039	602	**1010**	1012	1014				
1047	1048	702	1010	1011	1012	1014	**1025**	1049	

possessed by each offspring (Tables 3, 4 and 5). There were two additional offspring for which more than one male possessed all the paternally inherited alleles and therefore likely paternity could not be assigned to a single male. The 61 offspring for which no paternity could be assigned were conceived 1–12 years before sampling took place and thus we lacked DNA samples from many putative fathers residing within the study groups at the time of conception. The more recent the birth cohort, the greater the proportion of putative fathers that could be genotyped and the fewer offspring for which paternity could not be assigned (Fig. 2).

Aside from failing to sample the true father, we may have been unable to assign likely paternity to an offspring because either a mutation that changed the size of a paternally inherited allele or the presence of a null allele (nonamplifying) caused us to exclude falsely the true father of an offspring from paternity. The estimated rate of mutation at microsatellite loci indicates that we may have falsely excluded upwards of six actual fathers of offspring from paternity. Null alleles are not uncommon at microsatellite loci and can be detected in studies where parentage is not known a priori as observed deviations from Hardy–Weinberg expectations in the form of a heterozygote deficiency or loci which fail to amplify in particular individuals (Pemberton *et al*. 1995; Primmer *et al*. 1995). Although none of the loci we examined exhibited a heterozygote deficiency, six individuals failed to amplify at a particular locus, suggesting that they may have been homozygous for a null allele at that locus. The possibility of null alleles also means that individuals genotyped as homozygous may in fact be heterozygous for a null allele. Males which carry a nonamplifying allele which they pass on to their offspring, may be falsely excluded from paternity because the father and offspring can appear to be homozygous for different alleles at the locus with the null allele. Of the 61 offspring for which paternity could not be assigned, there were only two offspring where a male that was in the offspring's natal troop at the time of its conception was excluded from paternity solely on the basis of loci where both the offspring and the putative father were homozygous. Thus it seems unlikely null alleles resulted in the false exclusion of many true fathers.

Considering only those offspring born in the study groups between 1975 and 1986 for which a DNA sample was available, the mean number of males per year per group that fathered offspring did not increase significantly with the number of adult males present in the group (Fig. 4, $r = 0.41$, $P = 0.19$). It was not possible to assign paternity to all offspring born in every group each year over this period, particularly in the earlier years when DNA samples were not available from many of the potential fathers. However, it was possible to determine the minimum number of males required to father the offspring of unknown paternity in each group based on the number of different paternally inherited alleles present at

Table 5 Alleles present at each of the five loci used for paternity analysis for all individuals listed in Table 4. Dashes indicate no amplification at that locus

	Locus				
Individual	Mfd.125	Mfd.23	MFGT-17	MFGT-2	TRANS
101	AB	BB	DF	GG	CG
102	BB	DD	EF	BE	CG
103	BC	BD	DF	BF	GG
104	BB	BD	DF	EG	GG
105	BC	BD	DF	CG	GG
106	BD	BD	FF	BG	GG
107	BB	BD	DF	BG	CG
108	BD	BD	DF	GG	CG
109	BB	BD	FF	EG	CG
110	BC	BD	DF	BG	CG
111	BB	BD	FF	EG	GG
112	BD	DD	CF	GG	CG
113	BC	BD	DF	BG	GG
115	BD	BD	DF	EG	GG
201	CD	DD	FG	FG	DG
202	BB	DD	AC	BF	CG
203	BF	BD	AC	BG	CD
204	CF	DD	AE	AC	CD
205	FF	DD	AE	BB	DD
206	BD	DD	AG	FF	CG
207	CF	DD	AF	BF	DD
208	FF	DD	CD	AB	DD
209	CF	DD	EF	CF	DD
501	DE	BD	GG	BE	BD
502	EF	BD	FG	BB	BC
503	FF	BD	FG	BG	CD
504	DF	DD	CF	BC	DG
505	DF	DD	CF	BC	DD
506	BB	BD	AG	EF	CG
507	AF	BD	DG	FG	DD
509	BB	BD	AD	BF	CG
510	AF	BD	DD	CF	DG
511	AF	BD	DF	BC	DD
512	BF	BD	DG	BF	CD
513	BF	DD	FG	EG	CD
514	DF	DD	DF	BC	DD
515	BF	DD	DG	BF	CC
516	AD	DD	DE	CF	DG
517	DF	DD	FG	BB	DD
518	BF	BD	DG	BB	CC
520	BF	BD	AF	EG	CD
522	DF	BD	FG	BE	BD
523	DF	BD	DF	CG	CG
524	BF	BD	DG	BF	DG
525	BF	BD	GG	BF	CG
601	DD	BD	CG	BE	GG
602	BD	DD	FG	AE	BG
604	BB	BB	FF	EG	CG
605	BD	BB	FG	FG	CG
606	DD	BB	GG	EG	GG
607	BD	AB	DF	FG	CD
608	DD	BD	DF	AG	CG
609	BD	BD	DF	EG	CG
610	DD	BD	DF	GG	GG
611	DE	BD	DF	EG	CD
612	DD	DD	DG	AG	DG
613	BD	BB	DE	GG	DG
614	BB	BD	DF	AG	DG
616	BB	BB	FG	FG	CC
617	BD	BB	DF	AG	CD
618	BG	BD	GG	AG	CG
620	BB	BD	FG	EF	CG
701	BC	DD	FG	EF	GG
702	BG	AB	GH	EF	GG
703	BC	DD	FF	BE	CG
704	DF	BD	FG	AE	BD
705	BD	AB	EF	EG	CG
706	CD	BD	FF	EG	GG
707	CD	AD	GG	FF	GG
708	DD	DD	FG	FF	GG
709	BC	AB	FG	FF	GG
710	BG	AB	FF	EG	DG
711	DE	BD	GH	CE	CD
712	CD	BD	CF	AF	DG
713	CC	BD	CF	CF	GG
714	BD	BD	CF	AC	DG
715	BD	DD	FG	BF	GG
716	DD	BD	EF	EF	DD
717	BG	AB	EF	BE	DG
718	CD	AB	FF	EF	DG
719	DD	BD	FG	BC	GG
720	BB	BD	FI	GG	GG
721	BG	BD	FG	EG	CG
722	AD	BD	FF	BG	GG
723	BD	BD	GH	EF	CG
724	DG	BD	FG	EG	GG
725	DG	BD	AF	GG	DG
726	AD	BD	DG	FG	CC
801	BB	BD	FG	FG	CG
802	DD	BD	DF	EG	DG
803	BD	BD	FG	CE	CG
804	BD	DD	FF	AG	CD
805	BD	DD	FG	BE	GG
806	BD	DD	FF	EG	DG
807	BD	BD	DF	EG	DG
808	BG	BB	GG	EG	DG
809	BD	BD	FF	EE	CG
810	DE	BD	AC	EG	DG
811	BD	AB	- -	CG	DG
812	BG	BD	FG	CE	GG
813	BD	BD	HH	EG	CG
814	BD	BD	GH	FG	CG
815	AB	DD	FG	EG	CG
816	AB	AD	DH	BG	CG
817	AE	DD	HH	CG	CC
818	BG	DD	FG	CE	GG
819	AE	BD	GH	CG	CG
820	CD	BD	FG	AF	CG

Table 5 *Continued*

Individual	Locus				
	Mfd.125	Mfd.23	MFGT-17	MFGT-2	TRANS
821	BE	BD	FF	EG	GG
822	DE	BD	GG	EG	GG
824	EG	DD	FG	EG	GG
825	DF	BD	FF	CE	GG
826	BF	BD	FG	EG	DG
827	BE	BB	FG	BG	GG
828	BG	–	EF	BG	CG
829	DG	DD	DF	EF	CD
830	BE	BB	EF	BG	BG
831	BB	DD	FG	EF	CG
832	BG	BD	–	AG	DD
833	CD	BD	AF	EG	DG
835	DD	DD	FG	EG	CG
901	BG	BD	GH	AF	DG
902	BC	BD	FG	CE	CG
904	CC	BD	AH	BG	GG
905	BB	AB	FF	CC	GG
906	CD	DE	HH	CG	DG
908	BF	BB	FH	BC	DG
909	CD	BD	FG	CG	DD
910	CG	BD	FH	CF	CD
911	CG	BE	GH	EG	CD
912	CG	BD	AD	BE	CG
913	BH	BB	FH	CF	CG
914	CC	BE	DF	CE	CG
915	BC	DD	FH	BE	GG
916	CC	DE	DH	BC	DG
917	AD	DD	CH	CG	CG
918	CF	BD	FH	BB	CD
925	CD	DD	HH	AE	GG
926	CD	BD	GH	CE	BC
929	DJ	DD	GK	EG	DD
930	CJ	DD	GH	BG	DG
932	BD	DD	GG	BG	CC
933	DE	DD	GK	BG	CG
939	DJ	DD	GG	CG	DG
941	BD	DD	FH	GG	DG
942	BD	DD	AF	BF	GG
944	BD	DD	AH	FG	GG
951	DH	DD	FG	EG	DG
952	DG	BD	EG	EE	DD
953	AD	BB	EH	EF	GG
954	DD	BD	EF	EE	GG
955	DG	BB	AE	BG	DG
956	DD	DD	EH	FG	DG
957	DD	BD	EH	BF	DG
958	DD	AB	DF	EE	BD
959	DD	DD	DF	CG	DG
960	–	BD	DE	–	CG
961	–	AD	AK	AG	DG
962	CD	DD	AE	BG	DG
964	CD	AD	AG	EG	CG
966	BD	BD	FG	CC	BD
967	BD	BD	EH	BF	CD
969	CD	DD	FG	EG	GG

Table 5 *Continued*

Individual	Locus				
	Mfd.125	Mfd.23	MFGT-17	MFGT-2	TRANS
970	BG	DD	DF	EG	DG
972	BD	BD	AE	CE	BC
975	DF	BD	AG	CC	DG
977	DD	BD	AF	EF	GG
978	DF	DD	CG	EE	DG
979	AF	BD	AF	CE	DG
980	DD	BB	FG	CE	GG
981	AB	DE	DF	CE	CD
982	AD	DD	CF	EF	CG
985	DF	DD	AG	CE	DG
986	DG	DD	HH	EG	DG
988	DH	DD	DD	EF	GG
989	DF	BD	DH	EF	DD
990	DD	DD	GG	CF	GG
991	DF	DD	AG	EF	DG
992	BD	DD	EF	BF	CG
993	DF	DD	EG	BE	CD
994	AF	DD	FH	AF	DG
996	BB	BD	GH	AE	CG
997	FG	AD	GH	AE	DG
999	BB	BD	AH	BE	CC
1000	BC	BD	GH	AB	CG
1001	BD	DD	FH	AB	BC
1002	DF	DD	AF	AC	BD
1003	BB	BD	CF	BG	CC
1004	BF	BD	CG	BC	CD
1010	BC	DD	GH	CM	CG
1011	BB	DD	FG	EF	CG
1012	AF	BD	D I	AC	CG
1013	DD	BD	FG	CF	GG
1014	BD	DD	GH	BB	BC
1015	AD	BD	CH	BC	GG
1016	BD	DD	GH	AE	CG
1018	CF	BD	FJ	FG	DG
1019	DD	BD	GH	BE	CG
1020	BD	DD	GH	BE	GG
1021	DD	AB	AG	BF	DG
1022	BD	DD	GG	EF	CG
1023	CD	BD	GG	EG	CG
1024	CD	BD	CC	CE	CG
1025	BD	AA	CG	BC	DG
1026	BD	DD	AC	BC	CG
1027	DD	DD	CG	EF	BG
1028	DF	BD	DG	CF	CG
1029	DD	DD	GG	EF	GG
1030	BD	DD	HK	CE	CG
1031	BD	DD	HH	BC	BG
1032	BD	DD	GG	BC	CG
1033	BD	BD	CG	AB	GG
1034	AD	AD	CC	AM	GG
1035	BD	BD	CF	AC	CG
1036	CD	DD	GG	CE	CG
1037	CD	DD	CH	FM	CG
1038	CD	AB	CG	CF	CG
1039	BB	AD	GK	AC	CG

Table 5 *Continued*

	Locus				
Individual	Mfd.125	Mfd.23	MFGT-17	MFGT-2	TRANS
1040	BD	BD	FG	CC	CG
1041	AD	BD	AH	BG	DG
1042	CD	BD	CG	CE	GG
1044	CD	BD	GH	CE	GG
1045	CD	DD	GH	CE	DG
1046	BB	DD	DH	–	CG
1047	BB	AD	CK	AE	GG
1048	BB	AD	CK	BE	GG
1049	CD	DD	DG	CG	GG
1050	AD	DD	CF	BB	GG
1051	AD	AD	GK	CE	CG
1052	CD	BD	GH	CF	GG
1053	CD	DD	CG	CE	CG
1054	AD	BB	H I	CF	GG

each locus among these offspring. Therefore, the estimate of the number of males reproducing in each group per year represents a minimum estimate.

Among those males for whom we could assign likely paternity ($n = 77$), nine fathered offspring on two separate occasions with the same female and two fathered offspring on three separate occasions with the same female (Fig. 3). Of the 25 occurrences where a male who fathered an offspring by a particular female was still present in the social group when that female conceived again, that male also fathered the subsequent offspring on 13 (52%) occasions. In all seven of the cases in one-male groups where males remained after fathering an offspring and the female conceived again, the same male fathered the subsequent offspring, but in multimale groups the same male fathered a subsequent offspring by the same female in only six of 18 opportunities (33%). Because paternity determinations were done cross-sectionally on longitudinal demographic data, we lack paternity information on offspring that had died before DNA sampling. Therefore, these estimates of the temporal stability of mating partnerships between years are likely to be underestimated. There was no evidence that any of the 77 offspring for which we were able to assign likely paternity were the result of matings between first degree relatives.

There were 73 offspring (including the two offspring for which more than one male possessed all the paternally inherited bands) for which we were able to assign likely paternity to a male that was resident in an offspring's natal group at the time that it was conceived and six offspring for which we assigned likely paternity to a male resident in a social group other than the one in which the offspring was conceived. In addition, there were eight offspring for which we could exclude all of the resident males present at conception from paternity without being able to identify the nonresident father. Across all social groups, resident males were the likely fathers of 84% (73 of 87) of the sampled offspring. Resident males were the likely fathers of 17 of 23 (74%) offspring born in groups which contained only a single resident adult male at the time of the offspring's conception and 56 of 64 (88%) offspring born in groups with more than one resident adult male. These ratios were not significantly different ($\chi^2 = 1.42$, d.f. = 1, NS).

Paternal DNA was not sampled for seven additional offspring born in three different one-male groups. But within each group, all offspring shared the same paternal alleles, suggesting a single father, presumably the known single resident male, for all offspring per group. If these estimates of paternity are included, resident males were the likely fathers of 85% (80 of 94) of the sampled offspring across all groups and of 24 of 30 (80%) offspring born in a one-male group. The ratio of resident to nonresident paternity in this case is still not significantly different from that in multimale groups ($\chi^2 = 0.385$, d.f. = 1, NS).

The number of sampled males that fathered offspring in the 1986 birth cohort (birth season between 1 October 1986 and 30 September 1987), the birth cohort for which DNA samples are available from the greatest proportion (71%) of the resident males in the groups sampled, within a group is shown in Fig. 5. DNA samples were not available from all of the potential fathers because between conception of the offspring in this birth cohort and the time the samples were collected in 1987, some males died or dispersed out of the study groups. The number of offspring in the 1986 birth cohort fathered by each adult male for which a DNA sample was available when these offspring were conceived is shown in Fig. 6. Of the 27 offspring of the 1986 birth cohort for which we could assign likely paternity, three were fathered by males not resident in the offspring's natal group.

The relationship between the number of offspring a male produces within his resident group and the number

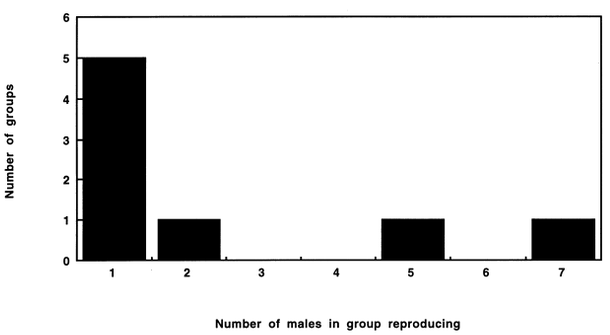

Fig. 5 The number of sampled males that fathered offspring in the 1986 birth cohort within each group that produced offspring in this birth cohort.

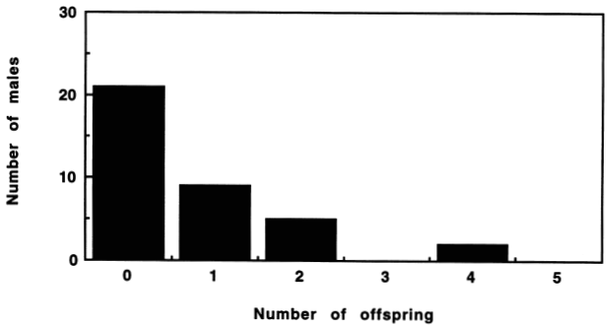

Fig. 6 The number of offspring in the 1986 birth cohort fathered by each adult male for which a DNA sample was available when these offspring were conceived.

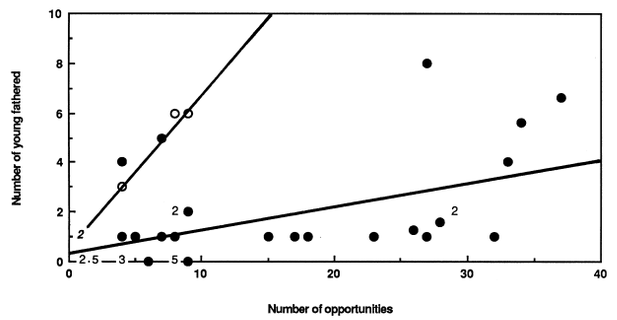

Fig. 7 The relationship between the number of offspring a male produced and the number of opportunities he had to father offspring in his resident group for all sampled males between 1975 and 1986. A male was considered to have had the opportunity to father an offspring if he was resident in an offspring's natal group at the time the offspring was conceived. Each circle represents a different genotyped male. Data for males in one male groups are represented by ○ ($n = 5$, $r = 0.99$, $P = 0.0003$) and by ● for males in multimale groups ($n = 40$, $r = 0.59$, $P = 0.0001$). The number in bold face italics indicates more than one male with the same value for males in one-male groups. Numbers in regular type indicate more than one male with the same value for males in multimale groups.

of opportunities he has had to father offspring is shown in Fig. 7 for all sampled males in one-male groups and in multimale groups between 1975 and 1986. The number of offspring a male fathered increased with the number of opportunities for males in one-male groups as well as for males in multimale groups but the slope of the increase for males in one-male groups ($r = 0.99$, $P = 0.0003$) was significantly greater than that for males in multimale groups ($r = 0.59$, $P = 0.0001$; $t = 4.45$, d.f. $= 41$, $P < 0.001$). Males in one-male groups fathered a much higher proportion of an offspring per opportunity than males in multimale groups. A male's proportion of an offspring fathered per opportunity decreased with the number of resident males in the group at the time the offspring was conceived

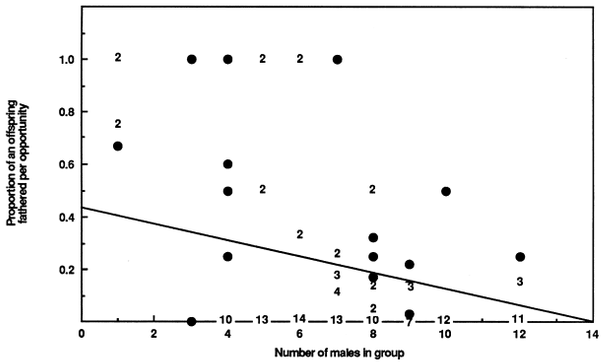

Fig. 8 The relationship between the proportion of an offspring fathered per opportunity and the number of resident males in the group at the time the offspring was conceived ($n = 138$, $r = 0.39$, $P = 0.0001$). Each circle represents an individual male's proportion of an offspring fathered when he was in a group containing a specific number of males when an offspring was conceived. Numbers indicate more than one male with the same value when in a group containing that many males.

(Fig. 8; $r = 0.39$, $P = 0.0001$). The more potential competitors for mating access to females tends to slightly reduce a male's reproductive success per opportunity.

Discussion

The potential for using microsatellite polymorphism as a means of determining paternity in free-ranging populations of primates is now well recognized (Berard *et al*. 1993; Takenaka *et al*. 1993; Morin *et al*. 1993). In this study we have been able to obtain statistically acceptable estimates of paternity for offspring of known maternity born into a free-ranging population of toque macaques, using genetic variation at four microsatellite loci in conjunction with that at a highly variable allozyme locus. This genetic analysis of paternity has in turn enabled us to elucidate some of the male patterns of reproduction in this population which could not have been ascertained by relying solely on the long-term demographic and behavioural data collected through observation.

Paternity of offspring born to the same mother

Although our assessment of the temporal stability of mating partnerships between years is likely to be underestimated due to the lack of DNA samples from offspring which disappeared prior to sampling, most of the males for which we could assign paternity (69%) fathered just a single offspring with a particular female. This suggests that there were few mating pairs which were stable over long periods of time and that the set of offspring that each female produces will usually consist of half-siblings and not be closely related through patrilineal relationships

(Altmann 1979). Knowledge of the level of relatedness within maternal sibships in macaque groups is crucial for evaluating the influence of relatedness on the pattern of altruistic/agonistic behavioural interactions observed among group members. The low frequency of male–female pairs that produce multiple offspring seems to be more a consequence of a lack of opportunity for a male–female pair to produce successive offspring due to a male's death or dispersal rather than to an active avoidance of remating. In fact, it was not uncommon for a male who fathered an offspring with a particular female to father a subsequent offspring as well if they were both still residing in the same social group, particularly when this situation occurred in a one-male group. The ability of some males to father multiple offspring with the same female may reflect a female's preference for that particular male or it may be due to that male possessing the characteristics necessary to monopolize access to the female through male–male competition during successive oestrus periods.

Inbreeding avoidance

Male dispersal coupled with a behavioural propensity to avoid consanguineous matings with close matrilineal kin are thought to minimize close inbreeding in cercopithicines (Dittus 1975; Melnick *et al*. 1984; Gouzoules & Gouzoules 1987). Because female toque macaques are philopatric and males disperse from their natal group upon reaching sexual maturity, opportunities for matings between mother and son or matrilineally related siblings are rare (Dittus 1979). However, because males may father young in groups in which they do not reside, the possibility of mother–son or sibling matings is not eliminated when a male disperses from its natal group. Of those offspring whose paternity could be attributed to a nonresident male, none was produced by matings between first-degree relatives. On the other hand, males may be present in their daughter's natal group when their daughters start to reproduce. Unfortunately, most of the females whose paternity we could establish had not yet reached the age of reproduction at the time the DNA samples were collected. Among those females whose paternity we could identify, only two had begun to reproduce. The fathers of both of these females were still resident in their daughter's group when their daughters conceived and the offspring of both of these females were apparently fathered by nonresident males.

Males may father offspring in more than one group because they may be resident in more than one group as a breeding adult or as a result of mating with nongroup females. Consequently, subsequent dispersal by their sons can create opportunities for matings between close paternal kin, which does not seem to be avoided in some other macaque species (Inoue *et al*. 1992). However, none of the males whose paternity was known dispersed into groups with reproductively active female paternal kin. Although our data revealed no evidence of close inbreeding (parent–offspring or sibling matings) among those offspring for which we were able to assign likely paternity, the number of instances where we could have detected close inbreeding were few ($n = 2$ for opportunities for father–daughter matings and $n = 0$ for opportunities for sibling matings). Only by following age cohorts of known paternity over several generations can the extent of inbreeding in this population be adequately evaluated. None the less, the lack of evidence in toque macaques is consistent with an analysis of genetic variation in a natural population of rhesus macaques in which groups exhibited an excess of heterozygotes, indicating an avoidance of consanguineous matings (Melnick *et al*. 1984).

Paternity by nonresident males

Our results indicate that the social unit in toque macaques is not identical to the reproductive unit and that the possibility of fertilizations by males not resident in an offspring's natal group should be taken into account when estimating male reproductive output. Similar results have been found in free-ranging groups of patas monkeys, where genetic data have shown that some offspring born in a social group must have been fathered by males that were not resident in the offspring's natal group at its conception (Ohsawa *et al*. 1993). These findings show that alternative mating tactics by males in these species can result in successful reproduction.

We should caution that our estimate of the proportion of offspring fathered by nonresident males is likely an overestimate. Estimated mutation rates at microsatellite loci suggest that in many of the cases ($n = 8$) where we were able to exclude from paternity all the resident males present in an offspring's natal group when it was conceived and therefore attributed likely paternity to an unidentified nonresident male, we may have in fact, falsely excluded the true resident father due to a mutation at one of the offspring's paternally inherited alleles. In addition, in four cases where likely paternity was assigned to a particular nonresident male, not all of the resident males present when that offspring was conceived were sampled. It is possible that one of the unsampled resident males may have been the true father and the nonresident male assigned likely paternity may have possessed the offspring's paternally inherited alleles by chance.

All the nonresident males to which we could assign likely paternity resided in multimale groups (range 5–8 males). Half of these males also fathered an offspring in the group they resided in during the same year they fathered an offspring in another group so attempting to reproduce as a nonresident male does not appear to be an

alternative strategy to reproducing in the male's resident group. One male that fathered an offspring as a nonresident had previously resided in the offspring's natal group. The group the male resided in and the offspring's natal group were originally a single group which had fissioned into two groups several years earlier.

In toque macaques, males which mate outside their group of residence might be expected to be more successful at fathering offspring in one-male groups because of less competition from the resident males, but our analysis showed that such outsiders were no more successful at fathering offspring in one-male groups than they were in multimale groups. One explanation for this result is that the success of nonresident males at fathering offspring may be more dependent on female choice than on the number of resident males in a troop monitoring the reproductive activities of females. In rhesus (Brereton 1981), Japanese (Wolfe 1986) long-tailed (van Noordwijk 1986) as well as toque (P. J. Dittus, unpublished data) macaques, some females seem to be attracted to nonresident males as mates.

Distribution of male reproductive output

While it was common for multiple males to reproduce each year within a social group, we could detect no significant effect of the number of adult males in a group on the average number of males reproducing per group per year. Cowlishaw & Dunbar (1992) found that the more adult males in a primate group the more difficult it was for a single male to monopolize access to oestrus females. Although in our study groups more males reproduced in groups with more adult males, the lack of a significant relationship between the number of males reproducing in a group and the number of adult males in a group may have been due to the small number of offspring born in some of the groups containing a large number of adult males. Within a social group, reproduction during a breeding season tended to be limited to a few males regardless of the number of adult males in the group (due in part to many groups producing only a few young per year). Examining the number of offspring produced by each male for which a DNA sample was available showed that during any given breeding season a few males were reproductively relatively successful while most males produce few or no offspring. Those males which were more successful during any one breeding season tended to be of higher social rank (W. P. J. Dittus *et al*. unpubl. data) but it was not possible to separate the relative importance of male–male competition from that of female choice. High social status may increase male reproductive output because high-ranking males are able to dominate physically lower ranking males for access to oestrus females and/or because those attributes responsible for high rank may also make those males attractive to females. Furthermore, because paternity within a social group is often limited to one or two males, age cohorts within a group may be more closely related by paternity than maternity (Altmann 1979).

While the reproductive output of individual males varied from year to year, our results suggest that males may also vary substantially in their lifetime reproductive success. Males in one-male groups had a significantly higher reproductive output per opportunity compared with the average male in a multimale group. This difference could potentially translate into a much greater lifetime reproductive output for males who lived for extended periods of time in a one-male group relative to that for the average male that lived his entire life in a multimale group. Within multimale groups, neither the number of opportunities a male had to father offspring in his resident group nor the number of males in an offspring's natal group when it was conceived were very good predictors of a male's likelihood of fathering an offspring. In particular, the lack of a strong relationship between the number of opportunities a male had to father offspring and the number of offspring fathered suggests that many males may father relatively few offspring during their entire life. It appears that many males lack the attributes and/or tactics necessary to successfully father many offspring. This skewed distribution of paternity as well as an already skewed male/female ratio at adulthood in favour of females (Dittus 1975) suggests a marked imbalance between female and male contributors to succeeding generations and thus a much smaller effective population size for toque macaques than would appear from simple demographic observations (Crow & Kimura 1970). Hence the potential for strong genetic drift effects and rapid genetic change may be enhanced in populations organized in this way (D. J. Melnick *et al*. unpubl. data).

Conclusions

This investigation illustrates how inferences about male primate mating patterns in nature can be greatly strengthened by coupling a genetic analysis of paternity with long-term demographic and behavioural data collected in the field. The emerging picture of male reproduction in toque macaques is a complex one in which males employ a variety of strategies to father offspring. Our results indicate that in the population of toque macaques at Polonnaruwa: (i) the set of offspring produced by a particular female tends to consist of half-siblings; (ii) none of the offspring whose likely paternity could be established was produced by matings between first-degree relatives; (iii) males may occasionally father offspring outside the social group in which they reside;

and (iv) during a breeding season, paternity within a social group tends to be limited to relatively few males. Genetic data on paternity in well-studied natural populations of primates, such as the toque macaques at Polonnaruwa, will not only permit the rigorous testing of numerous sociobiological hypotheses but will also provide important information for the conservation of threatened primates (Morin & Woodruff 1992; Morin *et al*. 1993).

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