

# Genetic Polymorphism in the Serotonin Transporter Promoter Region and Ecological Success in Macaques

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**Abstract** A well-characterised sequence length polymorphism in the serotonin transporter promoter region (5-HTTLPR) influences individual behavioural traits and cognitive abilities in humans and rhesus macaques. Macaques have been classified into four continuous grades on the basis of their behavioural attributes, ranging from highly hierarchical and nepotistic species to the most egalitarian and tolerant ones. A comparative study of several species that spanned these grades revealed only rhesus macaques to be polymorphic at the 5-HTTLPR and concluded that the polymorphism was responsible for their despotic and aggressive behaviour (Wendland et al., Behav Genet 36:163–172, 2006). We studied wild populations of three other species and found that the egalitarian and tolerant bonnet and Arunachal macaques are also polymorphic

while liontailed macaques, although belonging to the same group, are monomorphic. We thus reject a role for this particular polymorphism in interspecific behavioural variability and show that polymorphic species enjoy greater ecological success possibly due to their higher intraspecific variability in individual behavioural traits.

**Keywords** 5-HTTLPR · Bonnet macaque · Arunachal macaque · Liontailed macaque · Intraspecific variation · Behavioural flexibility

## Introduction

The serotonergic pathway, including the neurotransmitter serotonin and its transporter protein, is known to influence a wide range of individual behavioural traits and cognitive abilities in humans (Lesch et al. 1996; Lucki 1998; Canli and Lesch 2007). The serotonin transporter (5-HTT), which plays a key role in the intrasynaptic availability of serotonin, is encoded by a single gene, SLC6A4, the transcriptional activity of which is modulated by a repetitive length variation at the 5-HTT-linked polymorphic region (5-HTTLPR) within its promoter. A ‘short’ allele (*s*), with reduced transcription levels, appears to be partly responsible for certain anxiety-related personality traits; individuals homozygous or heterozygous for this allele thus exhibit increased neuroticism and reduced ‘agreeableness’ (Lesch et al. 1996; Canli and Lesch 2007), a higher risk of post-traumatic stress disorder and major depression (Caspi et al. 2003; Kilpatrick et al. 2007) as compared to those homozygous for the alternative ‘long’ allele (*l*). In rhesus macaques, an analogous length variation in the promoter region (rh5-HTTLPR) of this gene (Lesch et al. 1997) also influences neurobehavioural functioning; carriers of the

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short variant (*rh-s*) exhibit increased negative emotionality as infants, and poor control of aggression and stress reactivity throughout juvenile and adolescent development when deprived of maternal care during infancy (Champoux et al. 2002; Wendland et al. 2006).

Macaques are the most widely distributed and ecologically adaptable of all nonhuman primates, and exhibit remarkable variability in social organisation and individual behavioural strategies. The evolution of this diversity in behaviour and social structure, particularly within species, has been attributed to differences in ecology, demography and mating opportunities, while the variation across species is believed to be largely shaped by phylogenetic inertia from shared ancestry (Di Fiore and Rendall 1994; Chapman and Rothman 2009). A recent classification of macaque social organisations on the basis of interspecific variation in aggression, reconciliation, dominance, nepotism, socialisation and temperament has suggested four continuous grades, ranging from highly hierarchical and nepotistic societies (Grade 1: rhesus, Japanese and Taiwan macaques) through two intermediate categories (Grade 2: longtailed and pigtailed macaques; Grade 3: stumptailed, Barbary, liontailed, bonnet, toque, Tibetan and Assamese macaques), to the most egalitarian and tolerant organisations (Grade 4: Sulawesi macaques) (Thierry 2000). It was also suggested that the structure of these organisations is more closely shaped by phylogeny than by environment (Thierry et al. 2000) and that specific genetic polymorphisms may contribute to this variability in species-level behaviour (Wendland et al. 2006).

Accordingly, Wendland et al. (2006) investigated the variability in the promoter region (rh5-HTTLPR) of the serotonin transporter gene in seven species of macaques, belonging to all the four grades, and found a functional polymorphism in rhesus macaques alone. They thus concluded that macaque species with tolerant societies, relaxed dominance and high levels of conciliation were monomorphic for the length variation in this region while the highly aggressive and hierarchical species, following the demonstrated pattern in rhesus macaques, could be polymorphic. It should be acknowledged, of course, that the direct association of such phenotypes with this particular genetic polymorphism has only so far been tested in laboratory experiments with rhesus macaques but never in wild or captive populations of any macaque species.

Our studies on the demography and social behaviour of the bonnet macaque *Macaca radiata*, a species endemic to peninsular India, have revealed the coexistence of two forms of social organisation—a species-typical multimale–multifemale and a variant unimale–multifemale organisation (Sinha et al. 2005a). Adult males in these two kinds of societies, however, exhibit striking variation in their behavioural profiles, particularly with regard to aggression

directed towards adult and juvenile males, both within and outside their own social groups (Sinha et al. 2005a). The remarkable intraspecific behavioural variation displayed by bonnet macaques, which was originally classified a Grade 3 species (Thierry 2000), renders a species-based classification of macaque social organisation on the basis of certain limited behavioural traits and strategies, as was done by Thierry, overtly simplistic.

Given the contention of Wendland et al. (2006) that specific genetic polymorphisms, such as that in 5-HTTLPR, may contribute to variability in species-level behaviour across macaques, we examined the sequence length polymorphism at the 5-HTTLPR in bonnet macaques across its distributional range. The recently discovered Arunachal macaque from northeastern India (Sinha et al. 2005b) is closely related to the bonnet macaque and exhibits similar tolerant social relationships (Kumar et al. 2007). We, therefore, analysed the variability at the 5-HTTLPR in this species and also in the liontailed macaque, another endemic species restricted to the rainforest fragments of southern India and representing the Grade 3 group of macaques (Thierry 2000). It is noteworthy that this study is the first to examine the genetic variability at this important locus in macaque species, not only in the wild but also across their geographical range.

## Methods

### Study species

#### *Macaca radiata*

A total of 33 individuals of this species were sampled from different parts of its geographical distribution in peninsular India. Blood samples were obtained from 28 individuals of known origin, kept either in zoological parks or research laboratories, while skin/tissue samples were collected from five wild individuals that either died in road accidents or from natural causes.

#### *Macaca munzala*

Skin samples were obtained from the pelts of 24 individuals collected from the districts of Tawang, Upper Subansiri and West Siang in Arunachal Pradesh, northeastern India. Most of these individuals had been killed for food or sport and their pelts kept as trophies by the indigenous people of the region, while a few individuals were killed in retaliation for crop raiding.

*Macaca silenus*

Five skin samples were obtained from individuals that had died in road accidents and originally inhabited the Puthuthottam rainforest fragment of the Indira Gandhi Wildlife Sanctuary, Anaimalai Hills in Tamil Nadu state, southern India. The sixth individual was a wild-caught individual from Kerala, housed in a zoo, and from whom a blood sample was obtained.

It may be noted that none of the pelts, used in this study, were ancient but originated from individuals that had been killed relatively recently.

Kinship data were available for only nine individuals of the bonnet macaque (Table 1); such data were unavailable for the rest of the individuals in any of the species.

We hereby declare that we have complied with the guidelines for the care and use of laboratory animals as described by the U.S. National Institutes of Health whenever laboratory primates have been used to obtain blood samples.

## DNA extraction, amplification and sequencing

DNA was extracted from blood or skin/tissue of all the individuals using a DNeasy blood and tissue kit (Qiagen,

**Table 1** Genotype distribution and allelic variation at 5-HTTLPR in different populations of the three macaque study species

Species	Category of social organisation	Population	Genotype distribution			Allele frequency	
			LL	LS	SS	Long ( <i>l</i> )	Short ( <i>s</i> )
Bonnet macaque	Grade 3		15	14	4	0.67	0.33
<i>Macaca radiata</i> , <i>n</i> = 33		Surat, <i>n</i> = 3 <sup>a</sup>	3	0	0		
		Ahmadnagar, <i>n</i> = 2 <sup>b</sup>	2	0	0		
		Aurangabad, <i>n</i> = 1 <sup>c</sup>	0	0	1		
		Mumbai, <i>n</i> = 1 <sup>d</sup>	0	1	0		
		Pimpri, <i>n</i> = 3 <sup>e</sup>	0	2	1		
		Pune, <i>n</i> = 2 <sup>b</sup>	2	0	0		
		Bandipur, <i>n</i> = 4 <sup>f</sup>	1	3	0		
		Bangalore, <i>n</i> = 1 <sup>g</sup>	1	0	0		
		PRL Colony, <i>n</i> = 12 <sup>h</sup>	5	5	2		
	Thrissur, <i>n</i> = 4 <sup>i</sup>	1	3	0			
Arunachal macaque	Grade 3		23	1	0	0.98	0.02
<i>Macaca munzala</i> , <i>n</i> = 24		Tawang, <i>n</i> = 3 <sup>j</sup>	2	1	0		
		Upper Subansiri, <i>n</i> = 12 <sup>k</sup>	12	0	0		
		West Siang, <i>n</i> = 9 <sup>k</sup>	9	0	0		
Liontailed macaque	Grade 3		6	0	0	1.00	–
<i>Macaca silenus</i> , <i>n</i> = 6		Kerala, <i>n</i> = 1 <sup>i</sup>	1	0	0		
		Puthuthottam, <i>n</i> = 5 <sup>l</sup>	5	0	0		

<sup>a</sup> Wild-caught individuals, kept at the Dr. Shyamaprasad Mukharjee Zoological Garden, Surat, Gujarat state, western India

<sup>b</sup> Wild-caught individuals from Ahmadnagar, kept at the Rajiv Gandhi Zoological Park and Research Centre, Pune, Maharashtra state, western India

<sup>c</sup> Wild-caught individuals, kept at the Aurangabad Municipal Zoo, Aurangabad, Maharashtra state, western India

<sup>d</sup> Wild-caught individuals, kept at the Veermata Jijabai Bhosale Udyan and Zoo, Mumbai, Maharashtra state, western India

<sup>e</sup> Wild-caught individuals, kept at the Nisargakavi Bahinabai Choudhary Pranisangrahalay, Pimpri, Maharashtra state, western India

<sup>f</sup> Wild individuals, killed in road accidents, from the Bandipur National Park, Karnataka state, southern India

<sup>g</sup> Wild individual, which died from natural causes, from the GKVK Campus, University of Agricultural Sciences, Bangalore, Karnataka state, southern India

<sup>h</sup> Wild-caught and captive-born individuals from the Primate Research Laboratory, Indian Institute of Science, Bangalore, India; these included three related triads consisting of a mother, father and an offspring, each

<sup>i</sup> Wild-caught individuals, kept at the State Museum and Zoo, Thrissur, Kerala state, southern India

<sup>j</sup> Wild individuals, killed during crop-raiding by the local people across Tawang district, Arunachal Pradesh state, northeastern India

<sup>k</sup> Wild individuals, killed either for food or sport, or during crop-raiding by the local people across Upper Subansiri and West Siang districts, Arunachal Pradesh state, northeastern India

<sup>l</sup> Wild individuals, killed in road accidents, from Puthuthottam fragment, Indira Gandhi Wildlife Sanctuary, Tamil Nadu state, southern India

Hilden, Germany) following procedures recommended by the supplier, with the exception that DNA was incubated for 20 min in 100  $\mu$ l elution buffer before elution. The 5-HTTLPR sequence (potentially 640 bp) was amplified using a pair of oligonucleotide primers: rhMUT (5'-TCG ACTGGCGTTGCCGCTCTGAATGC-3'; Wendland et al. 2006; Miller-Butterworth et al. 2007) and stpr3 (5'-GAGGG ACTGAGCTGGACAACCAC-3'; Lesch et al. 1997; Miller-Butterworth et al. 2007). All the polymerase chain reactions (PCRs) were carried out in a final reaction volume of 10  $\mu$ l. The final concentration of the reaction mixture was: 5  $\mu$ l QIAGEN Multiplex PCR Master Mix (Qiagen, Hilden, Germany), 0.2  $\mu$ M of each primer (Ocimum Biosolutions, Hyderabad, India), 0.4  $\mu$ g/ $\mu$ l bovine serum albumin and 2.0  $\mu$ l of DNA. All the reactions were carried out in three Eppendorf Mastercycler (Eppendorf, Hamburg, Germany), one gradient and the other two non-gradient.

The amplification conditions consisted of an initial denaturation at 95°C for 15 min followed by a 50-cycle PCR to amplify the target segment with a denaturation of 30 s at 94°C, annealing of 30 s at 59°C, an extension time of 1 min at 72°C and with a final extension of 10 min at 72°C.

All the PCR products were resolved by running 2  $\mu$ l of each product in 2% agarose gels (Bangalore Genei, Bangalore, India), visualisation in a Gel Imaging System (Alpha Innotech, San Leandro, USA) and purified using Qiagen PCR purification kit (Qiagen, Hilden, Germany). The DNA concentration of the amplicons was measured by loading 2  $\mu$ l of the PCR products in a NanoDrop ND-1000 Spectrophotometer (Nanodrop Technologies, Delaware, USA). Bi-directional sequencing was accomplished using an ABI 310 Genetic Analyzer, and the raw sequences analysed with the ABI 310 Genetic Analyzer Version 3.1 software (Applied Biosystems, Foster City, USA). Sequences of variable lengths were edited manually and assembled using the software MEGA 4.1 (Tamura et al. 2007) and polymorphic regions were identified.

Positive and negative controls were employed at every stage of the extraction and amplification procedures wherever appropriate.

## Results

Sequence length polymorphism was observed at the 5-HTTLPR locus in the bonnet macaque *M. radiata* (HM114280-81) and the Arunachal macaque *M. munzala* (HM114278-79) (Table 1) while the liontailed macaque *M. silenus* (HM114282) was observed to be monomorphic at this region. The first two species exhibited heterozygosity for two allelic variants of the 5-HTTLPR, both of which were identical to those in the rhesus macaque (Lesch et al. 1997). The longer *l* allele for the three species

examined was analogous to that in the rhesus macaque and the longtailed macaque *Macaca fascicularis* (Fig. 1; Miller-Butterworth et al. 2007). The shorter *s* allelic variant appears to have been generated from a probable deletion of two repetitive motifs (of length 22 and 21 bp, respectively) at the second polymorphic locus, PL2, of 5-HTTLPR (Fig. 1; Lesch et al. 1997). No polymorphism was observed at the third polymorphic locus, PL3, in the bonnet macaque and liontailed macaque; these sequences were thus identical to those in the rhesus and longtailed macaques (Fig. 1; Lesch et al. 1997; Miller-Butterworth et al. 2007).

Our results also reveal that the polymorphism at the 5-HTTLPR locus is widespread across different populations of the bonnet macaque, which is in contrast to the Arunachal macaque, where the polymorphism is restricted to a single population out of the three tested (Table 1). In the single colony population of bonnet macaques tested (Table 1), we analysed three families of known parentage to determine the mode of inheritance of this polymorphism. Our results confirmed that the alleles at this locus indeed exhibit patterns of Mendelian inheritance.

## Discussion

Bonnet macaques have been categorised as an egalitarian Grade 3 species (Thierry 2000) while the Arunachal macaque, which belongs to the same *sinica* species-group and is characterised by tolerant social relationships (Kumar et al. 2007), is likely to belong to the same grade. Our sequence data revealed that these two species share the same 43-bp deletion with rhesus macaques (Lesch et al. 1997), clearly ruling out the possibility of the sequence length polymorphism at the 5-HTTLPR being responsible for the behavioural profiles displayed exclusively by the highly hierarchical and aggressive Grade 1 species such as the rhesus macaque (Wendland et al. 2006). Our study, in fact, raises important questions regarding the validity of Thierry's (2000) classification scheme; the levels of intraspecific variation in behavioural profiles displayed by wild bonnet macaques (Sinha et al. 2005a) would itself warrant a critical re-examination of a behavioural classification of macaque social organisation solely based on broad interspecific variation in select behavioural traits.

Although specific genetic polymorphisms could indeed contribute to inter-specific variability in macaque social behaviour, we are of the opinion that this particular length polymorphism at the 5-HTTLPR is more likely to be involved in within-species variation in macaque behavioural profiles.

A notable example of such intraspecific behavioural variation in bonnet macaques is our recent observation of two distinct forms of social organisation that stably co-occur within a certain population of this species in the Bandipur

**Fig. 1** Multiple sequence alignment of 5-HTTLPR alleles from six species of macaques. For alignment optimisation and comprehensibility, hyphens have been introduced and 10-bp units separated by spaces. Mti: single allele of *Macaca thibetana* (GenBank Accession Number AY897213); Mmu S, L and XL: ‘short’, ‘long’ and ‘extra long’ alleles of *M. mulatta* (AF191557); Mfa: single allele of *M. fascicularis* (EF126284); Msy: *M. sylvanus* allele (AY897212); Mra S and L: ‘short’ and ‘long’ alleles of *M. radiata* (this study; HM114280-81); Mmz S and L: ‘short’ and ‘long’ alleles of *M. munzala* (this study; HM114278-79); Msi: single allele of *M. silenus* (this study; HM114282). Note that PL2 and PL3 (highlighted in the figure) represent the two polymorphic loci within the 5-HTTLPR in macaques. Complete sequence information is not available for the Mmz S and L alleles

Mti	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCC-AT	60	
Mmu S	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CAACCCCCAT		
Mmu L	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CAACCCCCAT		
Mmu XL	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CAACCCCCAT		
Mra S	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCCAT		
Mra L	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCC-AT		
Mfa L	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCCAT		
Mmz S	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCC-AT		
Mmz L	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCC-AT		
Msi	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCCAT		
Msy	TGCTGCAGCC	CTCCCAGCAT	CTCCCTGTAC	CCCTCCTAGG	ATCTCCCCTG	CACCCCCAT		
				<b>Polymorphic Locus 2 (PL2)</b>				
Mti	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----	-----	120	
Mmu S	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----	-----		
Mmu L	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Mmu XL	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Mra S	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----	-----		
Mra L	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Mfa L	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Mmz S	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----	-----		
Mmz L	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Msi	TATCCTCCCT	ACACCCCCCA	--GCAT	-----	-----CCCC	CCTGCAGCCT		
Msy	TATCCTCCCT	ACACCCCTTG	CGGCAT	CCCC	CCTGCACCCC	CAACATCCCC	CCTGCAGCCT	
				<b>PL2</b>				
Mti	-----	-----	-----	C	CCCCCTGCAG	CCCTTTCAGT	ATCCCCCTGC	180
Mmu S	-----	-----	-----	C	CCCCCTGCAG	CCCTT-CAGC	ATCCCCCTGC	
Mmu L	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTT-CAGC	ATCCCCCTGC	
Mmu XL	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTT-CAGC	ATCCCCCTGC	
Mra S	-----	-----	-----	C	CCCCCTGCAG	CCCTTTCAGC	ATCCCCCTGC	
Mra L	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTTTCAGT	ATCCCCCTGC	
Mfa L	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTTTCAGC	ATCCCCCTGC	
Mmz S	-----	-----	-----	C	CCCCCTGCAG	CCCTTTCAGT	ATCCCCCTGC	
Mmz L	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTTTCAGT	ATCCCCCTGC	
Msi	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTTTCAGC	ATCCCCCTGC	
Msy	CCCAGCATCT	CCCCTGCACC	CCCAGCATC	C	CCCCCTGCAG	CCCTTTCAGC	ATCCCCCTGC	
Mti	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC	240	
Mmu S	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mmu L	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mmu XL	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mra S	ACCCCTCCCA	GGATCTCCCT	TGCATGCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mra L	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mfa L	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mmz S	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Mmz L	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Msi	ACCCCTCCCA	GGATCTCCCT	TGCATCCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		
Msy	ACCCCTCCCA	GGATCTCCCT	TGCA-CCCCA	TTATCTCCCC	TGCACCCCTC	GCAGTATCCC		

National Park-Mudumalai Wildlife Sanctuary complex, southern India, with the resident adult males of the two organisations displaying markedly different levels of aggression-related behaviour (Sinha et al. 2005a). In the unimale-multifemale organisation, the single adult male enjoys a complete reproductive monopolisation of the troop females and this appears to be related to a strong intolerance of other males, both within and outside the group. The several behavioural manifestations of this intolerance, including severe aggression towards subadult and juvenile males within the group, herding of the group females, complete involvement in group defense against other groups, and active prevention of male immigration into the group, appear to be characteristic of these males and are only sporadically displayed by the adult males in the alternative multimale-multifemale form of social organisation (Sinha et al. 2005a).

An ecological factor that may have significantly affected bonnet macaque populations in the recent past is the increasing dependence of many of these populations on provisioned food (Kurup 1981; D’Souza and Singh 1992; Sinha 2001). Such food is often nutritionally rich and provisioning is thus usually marked by intense contest competition within troops, and scramble and contest competition among neighbouring troops. This is accentuated during the dry summer months when natural food resources are particularly sparse and very patchily distributed, and most of the troops become more dependent on provisioned food. The amount of provisioned human food, however, is also rather unpredictable and clumped in distribution, and like the natural food sources of the dry season, is usually able to support small groups of macaques (Sinha et al. 2005a). Bonnet macaque females are typical of



Fig. 1 continued

<b>Mt i</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCC-	TTGCGGCATC	CCCCCTGCA	CCCC-AGTA	300
<b>Mmu S</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TCGCGGCATC	CCCC-TGCA	CCCTCCAGTA	
<b>Mmu L</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TCGCGGCATC	CCCC-TGCA	CCCTCCAGTA	
<b>Mmu XL</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TCGCGGCATC	CCCC-TGCA	CCCTCCAGTA	
<b>Mra S</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCCCCCAGTA	
<b>Mra L</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCCCCCAGTA	
<b>Mfa L</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCCCCCAGTA	
<b>Mnz S</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCCCCCAGTA	
<b>Mnz L</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCC	
<b>Msi</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGAATC	CCCC-TGCA	CCCCCCAGTA	
<b>Msy</b>	CCCGCACCTC	CATTATCCCC	CCTGCACCCC	TTGCGGCATC	CCCC-TGCA	CCCCCCAGTA	
<b>Mt i</b>	TTCCCCCTGG	AGCCCCCCCC	AGCATCTCCC	C-GCACCAGAC	ACAGCATCCC	CCCTGCAGCC	360
<b>Mmu S</b>	TTCCCCCTGC	AGCACCCCCC	AGCATCTCCC	CCGCACCGCC	C-AGCATCCC	CCCTGCAGCC	
<b>Mmu L</b>	TTCCCCCTGC	AGCACCCCCC	AGCATCTCCC	CCGCACCGCC	C-AGCATCCC	CCCTGCAGCC	
<b>Mmu XL</b>	TTCCCCCTGC	AGCACCCCCC	AGCATCTCCC	CCGCACCGCC	C-AGCATCCC	CCCTGCAGCC	
<b>Mra S</b>	TTCCCCCTGC	AGCCCCCCCC	AGCATCTCCC	CCGCACCGCT	C-AGCATCCC	CCCTGCAGCC	
<b>Mra L</b>	TTCCCCCTGC	AGCCCCCCCC	AGCATCTCCC	CCGCACCGCT	C-AGCATCCC	CCCTGCAGCC	
<b>Mfa L</b>	TTCCCCCTGC	AGCACCCCCC	AGCATCTCCC	CCGCACCGCC	C-AGCATCCC	CCCTGCAGCC	
<b>Mnz S</b>	TTCCCCCTG						
<b>Mnz L</b>							
<b>Msi</b>	TTCCCCCTGC	AGCCCCCCCC	AGCATCTCCC	CCGCACCGCT	C-AGCATCCC	CCCTGCAGCC	420
<b>Msy</b>	TTCCCTCTGC	AGCCCCC--	AGCATCTCCC	CCGCACCGCC	C-AGCATCCC	CCCTGCAGCC	
<b>Polymorphic Locus 3 (PL3)</b>							
<b>Mt i</b>	CTT-----	-----	-----	CCAA	GCAACCCCT	GCAT	464
<b>Mmu S</b>	CTT-----	-----	-----	CCAG	GATCTCCCCT	GCAA	
<b>Mmu L</b>	CTT-----	-----	-----	CCAG	GATCTCCCCT	GCAA	
<b>Mmu XL</b>	CTTCCAGCAA	CCCCCTTGCA	TCCCTC	CCAG	GATCTCCCCT	GCAA	
<b>Mra S</b>	CTTCCAGCAA	CCCC-TGCA	TCCCTC	CCAG	GATCTCCCCT	GCAA	
<b>Mra L</b>	CTTCCAGCAA	CCCC-TGCA	TCCCTC	CCAG	GATCTCCCCT	GCAA	
<b>Mfa L</b>	CTTCCAGCAA	CCCC-TGCA	TCCCTC	CCAG	GATCTCCCCT	GCAA	
<b>Mnz S</b>							
<b>Mnz L</b>							
<b>Msi</b>	CTTCCAGCAA	CCCC-TGCA	TCCCTC	CCAG	GATCTCCCCT	GCAA	
<b>Msy</b>	CTT-----	-----	-----	CCCA	GCAACCCCT	GCAT	

cercopithecine primates in being philopatric and thus form small, stable, core groups in such areas where food resources are either deficient in the dry season or are provisioned to a considerable extent. These groups are then often easily taken over by a single dominant adult male, who is able to successfully prevent reproductive access of other males to the group females and thus effectively maintain the unimale–multifemale form of social organisation in this population (Sinha et al. 2005a).

It is quite possible that certain epigenetic factors such as phenotypic flexibility (Sinha 2005) could lead to the observed behavioural variation displayed by male bonnet macaques in this population. It is, however, also entirely conceivable that polymorphisms at genetic loci, such as 5-HTTLPR, which impart behavioural plasticity to individuals, could enable certain males with specific genotypes to be able to aggressively take over the relatively smaller, recently formed, groups of females and monopolise reproduction in such groups, thus allowing for specific alleles to be selected for in such situations. We propose to examine genetic variability across individual macaques and compare allele frequencies across these two types of social organisations at the 5-HTTLPR and other related loci in the near future. Any conclusion that may be drawn regarding genetic selection in such contexts should, however, be tempered by the fact that small allelic differences in

genotypes could also often reflect neutral processes such as genetic drift rather than directional selection.

It may also be argued that similar ecological variability may present itself, even without anthropogenic causes, when macaque populations colonise new habitats with differing patterns of resource abundance and distribution. The remarkably ubiquitous presence of the bonnet macaque and its wide distribution across a great diversity of habitats in peninsular India (Sinha 2001), not always necessarily in close proximity to human populations, could thus possibly be attributed to this social flexibility and the ability of individuals to adopt alternative behavioural strategies in different contexts (Sinha 2005). As outlined above, underlying genetic polymorphisms could, of course, contribute to such within-species behavioural variation.

It is noteworthy that our data reveal a very comparable frequency of the *l* and *s* alleles across bonnet (*l*: 0.67, *s*: 0.33) and rhesus macaques (*rh-l*: 0.74, *rh-s*: 0.26; Wendland et al. 2006). The latter species also exhibits significant ecological adaptability, behavioural flexibility, and has the largest geographical distribution amongst all nonhuman primates (Maestriperi 2007). The remarkable ability of both these species to adapt successfully to a wide range of ecological regimes, many of which are of an anthropogenic nature, has thus earned them the sobriquet ‘weed macaques’ (Richard et al. 1989). Suomi (2006) has, in fact,

already speculated that the ecological success of both humans and rhesus macaques may derive not from “exquisite” genetic specialisation but instead from more general genetic *variation* (italics his) including that at the 5-HTTLPR. In other words, these species may have been immensely successful in colonising new habitats not because they have been able to specialise in these environments but because they have acquired the genetic predisposition to be much more flexible in their social organisations and behavioural profiles. Our data on the bonnet macaque provides clear support to this hypothesis although it should be noted that we have not examined the behavioural profiles of the individuals that have been genetically sampled during this preliminary analysis. On the same note, a recent review by Belsky et al. (2009) suggest that such genes that make individuals disproportionately vulnerable to psychosocial adversity such as stress or depression (Lesch et al. 1996; Champoux et al. 2002; Caspi et al. 2003; Canli and Lesch 2007) may simultaneously confer on them an advantage when dealing with certain types of environmental variation and these genes should therefore also be considered as ‘plasticity genes’ and the individuals bearing them as ‘malleable’ rather than just ‘vulnerable’ to environmental changes. We believe that such plasticity manifests itself strongly in socioecological contexts that highly ecologically adaptable macaques, including the weed macaques, encounter regularly during their movement and range expansion.

It is perhaps significant that both rhesus macaques and bonnet macaques, which exhibit high levels of diversity in aggression-related behavioural traits, are also polymorphic at the 5-HTTLPR of the serotonin transporter gene, although our study was not designed to investigate the causal nature of this genotype–phenotype association. It is conceivable, nevertheless, that such a polymorphism, even if it did arise in a population, may not have spread in species such as the Barbary (Wendland et al. 2006) or the liontailed macaque (this study), which are restricted to narrow, homogeneous ecological regimes without much opportunity to increase their geographical range further. Variation in individual behavioural profiles may thus not confer any significant advantage to individuals bearing such mutations in these species. Such a conclusion is also supported by our data on the Arunachal macaque, in which species the *s* allele occurs at a very low allele frequency, its occurrence being restricted to a single population out of the three examined. Populations of this species occur at fairly high altitudes of the Eastern Himalayas and are completely geographically isolated from each other (Sinha, unpublished data). Geography thus plays a crucial role in preventing the expansion of such populations and this, in turn, could severely restrict the importance of genetic polymorphisms like the one at 5-HTTLPR, which confers behavioural flexibility, in these

populations. Thus, in the wild populations of the three different macaque species that we investigated, the presence or absence of this particular genetic polymorphism is strongly linked to their ecology and geography. In the bonnet macaque and the liontailed macaque, therefore, the underlying ecology of the species shapes their genetic variation whereas in the Arunachal macaque, which shares a close common ancestry with the bonnet macaque (Chakraborty et al. 2007), geography serves to restrict such variability though populations may have inherited particular polymorphisms through phylogenetic inertia.

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