ABSTRACT

Title of Document: ASOCIAL MONOGAMY, EXTRA-PAIR PATERNITY, AND DISPERSAL IN THE LARGE TREESHREW (*Tupaia tana*)

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Monogamy occurs in only 5% of mammalian species, but is significantly more common in the Euarchonta: primates, dermopterans, and treeshrews (15% spp.). However, many of these species do not breed monogamously, indicating the need to understand behavioral and genetic monogamy as separate evolutionary phenomena. I examined monogamy in the large treeshrew (*Tupaia tana*) in Sabah, Malaysia using radiotelemetry data from 46 individuals tracked during and after a fruit masting episode in 1990-1991, during a non-masting period from 2002-2004, and in a selectively logged forest from 2003-2004. I show that large treeshrews exhibit behavioral monogamy in all these ecological situations. However, behavioral monogamy is best characterized as dispersed pair-living, or "asocial monogamy", in this species because male-female pairs travel, forage, and sleep alone on their joint territories.

Next, I use microsatellites and mitochondrial DNA d-loop haplotypes to analyze the genetic maternity and paternity of 24 *T. tana* offspring. I show one of the highest rates of extra-pair paternity (EPP) ever recorded for a behaviorally monogamous mammal. Over 40% of young were sired by males that were not the behavioral partner of their mother, and three litters exhibited evidence of multiple paternity. Comparative analysis of relative testis size in treeshrews and primates indicates that sperm competition is not associated with the high rates of EPP in *T. tana*, and that the evolution of monogamy is associated with the evolution of smaller testes.

Finally, I find genetic evidence of female-biased dispersal and gene flow in large treeshrews. The vast majority of mammals exhibit the behavioral combination of polygyny and male-biased dispersal, but female-biased dispersal may evolve in monogamous species when females compete for ecological resources. In support of the local resource competition hypothesis, I find lower population assignment probabilities and pairwise relatedness for females than males. These results indicate that female *T. tana* are a mixture of philopatric residents and immigrants from other areas. Coalescent-based Bayesian analyses also show that historical female migration has been three times higher than the overall migration rate between primary and logged forest populations, providing evidence of female-biased gene flow.

ASOCIAL MONOGAMY, EXTRA-PAIR PATERNITY, AND DISPERSAL IN THE LARGE TREESHREW (*Tupaia tana*)

By

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Preface

This dissertation contains a single introduction section and three chapters. Chapters I, II, and III are presented in manuscript form, with abstract, introduction, methods, results, and discussion, followed by tables, figure legends, and figures. A single appendix follows the chapters in the format in which it was published (*Molecular Ecology Notes*. 2006. in press). A single bibliography section occurs at the end for references cited throughout the dissertation.

Dedication

To Versha, with love

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CHAPTER III

INTRODUCTION

Mating systems are the patterns of male-female associations within populations and have fundamental consequences for the evolution of behavioral and morphological traits (Shuster & Wade 2003). Animal mating systems encompass mate acquisition, the numbers of mates acquired, and the presence and characteristics of pair bonds within a population (Emlen & Oring 1977; Davies 1993). The form and duration of parental care has traditionally been considered a component of mating systems (Reynolds 1996; Trivers 1972), but recent studies suggest that parental care and mating systems do not consistently covary in many taxonomic groups (Fromhage et al. 2005; e.g. mammals, Komers & Brotherton 1997).

Substantial progress in understanding the diversity of animal mating systems followed the proposal that specific mating systems are the outcome of ecological constraints on male monopolization of reproductive females (e.g. bats and ungulates, Bradbury & Vehrencamp 1977; Emlen & Oring 1977). The key elements of this predictive framework are 1) the ecological factors (particularly predation and resource dispersion) influencing group size, dispersion, and ranging behavior of females, and 2) the intensity of reproductive competition between males mediated by the spatial and temporal distribution of receptive females. This view conceptualizes mating systems as the outcome of the aggregate behavior of individuals, and predicts that mating systems vary both within and between populations due to differences in the social and ecological environment (Clutton-Brock 1989).

Mating systems that may produce extremely high or low variance in male reproductive success, such as lekking or harem defense where one or a few males

monopolize reproductive opportunities (Höglund & Alatalo 1995; Andersson 1994), or *monogamy* where both females and males have only a single opposite-sex mate at a time (Gowaty 1996; Kleiman 1977; Reichard & Boesch 2003), have received particular attention from researchers. Monogamy is thought to evolve when paternal care is necessary to successfully raise offspring (Type I, or obligate, monogamy, Kleiman 1977; Clutton-Brock 1989), or when males can monopolize only a single female because females are solitary and highly dispersed (Type II, or facultative, monogamy, Emlen & Oring 1977). However, Trivers (1972) predicted that selection should favor those males that pair with a female to raise offspring, but also copulate and reproduce with extra-pair females. Support for this prediction arrived as detailed field studies revealed substantial extra-pair copulations in putatively monogamous species (e.g. birds, Westneat et al. 1990; gibbons, *Hylobates* spp., Reichard 1995; elephant shrews, *Elephantulus rufescens*, Rathbun 1979). The subsequent development and use of molecular markers to reveal that monogamy actually subsumes a diverse range of genetic mating strategies is one of the most important advances in behavioral ecology in the last 20 years (Bennett & Owens 2002; Hughes 1998).

The prevalence of monogamy among avian species and the relative logistical ease of monitoring reproduction at nests have made birds the favored organisms for genetic parentage analyses. Birds typically live in "monogamous" male-female pairs (> 90% spp., Lack 1968), but over 150 genetic studies have shown that most of these species also exhibit significant rates of extra-pair paternity (> 5% EPP in 112 of 130 species, Griffith et al. 2002). These studies have also indicated that females play a much larger role in monogamous mating systems than previously appreciated, as they may seek extra-pair

paternity to obtain good genes (e.g. Sheldon et al. 1997) or increase the genetic diversity of their offspring (e.g. Foerster et al. 2003). Alternatively, females may avoid extra-pair copulations to ensure the fidelity and parental investment of their mate (Arnqvist & Kirkpatrick 2005).

Preliminary results from mammals also indicate that the "monogamy" classification conceals diverse mating patterns. Fewer than 5% of mammals have been described as monogamous, although the percentage is considerably higher among the Euarchonta (primates, treeshrews, and dermopterans; 15% spp.), rodents, canids, and bats (Kleiman 1977; McCracken & Wilkinson 2000). Genetic parentage analyses have been conducted on only 10 of these mammalian species to date, but in six cases more than 10% of offspring were sired by extra-pair males (e.g. 44% EPP in the fat-tailed dwarf lemur, *Cheirogaleus medius*, Fietz et al. 2000). However, fundamental reproductive differences between birds and mammals, such as lactation, internal gestation, and a general lack of paternal care in mammals (Kleiman & Malcolm 1981; Komers & Brotherton 1997), suggest that the ecological and social causes of pair-living and EPP may differ between these two groups.

Discord between behavioral and genetic studies of monogamy have caused confusion over terminology and the relationship of social organization to mating systems in mammals. The dominant view among behavioral ecologists now asserts that the behavioral, sexual, and genetic components of monogamy must be examined separately to describe accurately mating behavior in "monogamous" species (Gowaty 1996; Fuentes 1999). Following Reichard (2003), *behavioral monogamy* refers to a close spatial and behavioral relationship between a male and female (e.g. may include defense of a joint

territory, affiliative / pair bonding behaviors, and / or proximity between partners), and *sexual monogamy* refers to exclusive copulation and other sexual behaviors between a male and a female. *Genetic monogamy* is reserved for cases where genetic parentage analyses confirm mating exclusivity between a male and a female (e.g. two *Peromyscus* spp., Ribble 2003). *Social monogamy* has often been used in place of behavioral monogamy (e.g. Gowaty 1996), but I use the latter term to avoid conflating mating systems, social organization, and social behavior, because social monogamy does not encompass species that do not exhibit substantial pair-bonding or social behavior.

The ecological factors influencing variation in behaviors comprising monogamy, and the relationship between behavioral monogamy and genetic mating systems, remain poorly understood for mammals (Kappeler & van Schaik 2002). Fuentes (2002) did not find a strong association between behavioral and genetic monogamy in primates, but few studies have measured paternity in the field. Monogamy also occurs in diverse forms among mammalian taxa. One set of species may live in multi-male and / or multi-female groups, but a single dominant pair is still the main reproductive unit (e.g. callitrichids, Dunbar 1995; Goldizen 1990). These societies often involve a complex system of reproductive suppression of subordinates and are probably best understood using reproductive skew theory (Beekman et al. 2003; e.g. African wild dogs, *Lycaon pictus*, Creel 2001). This study focuses on species where male-female pairs, and not extended families or social groups, are the dominant form of behavioral mating system. Behavioral monogamy in these species varies from *associated pairs* that remain cohesive in space and time on a common territory, to *dispersed pairs* that may share a territory but travel, forage and sleep alone (Kappeler & van Schaik 2002; van Schaik & Kappeler

2003). Additional studies of all components of monogamy, and the consequences of monogamy for other behaviors, are clearly needed for a complete understanding of mating systems in mammals.

The same ecological factors that Emlen and Oring (1977), and later, Shuster and Wade (2003), predicted would drive the evolution of monogamy are likely to act as selective pressures on dispersal patterns. In monogamous species without paternal care, females often occupy exclusive territories and are highly dispersed in space and time due to a relative scarcity of resources. Increased aggression and competition between females for feeding territories are likely to occur in these species, because food resources may be a primary determinant of variance in reproductive success among females. Greenwood (1980) predicted that this scenario would lead to female-biased dispersal as female juveniles either left voluntarily to find unoccupied territories, or were forced off their natal territory due to foraging competition with adults. In support of this prediction, behaviorally monogamous bird species primarily exhibit female-biased dispersal (Greenwood & Harvey 1982). Polygynous mammal species exhibit the opposite pattern of male-biased dispersal, most likely due to inbreeding avoidance or local competition between fathers and sons for mates (Dobson 1982; Perrin & Mazalov 1999). Monogamous mammals that exhibit local resource competition between females are predicted to exhibit female-biased dispersal, but evidence of this behavioral combination has proved elusive (Dobson 1982; Wolff 1994). However, advances in molecular genetics have overcome previous logistical difficulties (Goudet et al. 2002), and now facilitate robust detection of sex-biased dispersal in mammals (Favre et al. 1997; Hammond et al. 2006).

My dissertation research is the first comprehensive analysis of the mating system and dispersal patterns of a mammal from the order Scandentia: the large treeshrew, *Tupaia tana* (Figure 1). Treeshrews are one of the closest living relatives of primates (Murphy et al. 2001), and were long considered primitive members of that order (e.g. Simpson 1945). Treeshrews are not closely related to the order Insectivora, and I adopt Emmons' (2000) use of the single word *treeshrew* rather than "tree shrew" to distinguish them from the true shrews. Hundreds of laboratory studies were conducted on treeshrews during their period of glory as the primate outgroup (Elliot 1971), but research interest waned after they were placed in their own order (Luckett 1980). However, the new millennium may be considered the beginning of a treeshrew renaissance (Sargis 2004), as major studies of the behavioral ecology and natural history (Emmons 2000), genetics (Schmitz et al. 2000), molecular systematics (Han et al. 2000), and morphology (Sargis 2000) of treeshrews have recently been published. The order Scandentia is currently undergoing a long-needed systematic revision (Olson et al. 2004; Olson et al. 2005), and the sequencing of the treeshrew genome is underway (National Human Genome Research Institute, http://www.genome.gov/10002154).

Understanding mating systems of treeshrews is important because of their close phylogenetic relationship to monogamous primates, and the prevalence of monogamy among the order Scandentia (100% behavioral monogamy in 19 spp. from 2 families, Wilson & Reeder 1993). *Tupaia tana* is a small (200-250 g), diurnal mammal that inhabits the lowland rainforests of Borneo and Sumatra, and has been described as behaviorally monogamous based on spatial concordance between the territorial boundaries of male-female pairs (Emmons 2000). Treeshrews have historically been

considered insectivorous, but Emmons (1991) showed that fruit is an important component of the diet of *T. tana* and three other sympatric tupaiids. Over 25% of scats from captured *T. tana* contained fruit (excluding trap baits) in her study, despite gut transit times of only 38 minutes (Emmons 2000). Several lines of evidence suggest that fruit abundance influences female reproduction, and thus may underlie variation in the behavioral mating system of large treeshrews if fruit abundance also influences the space use and ranging behavior of females. Wild *T. tana* females typically give birth one to three times a year, but they exhibit postpartum estrus and are capable of reproducing nine times annually in captivity if fed fruit *ad libitum* (Emmons 2000). Wild and captive females also exhibit a unique, energetically-costly maternal care system, whereby females deposit their young in a secluded nest that they subsequently visit only once every 48 hours for intense bouts of nursing (Emmons 2000; Martin 1966). *T. tana* individuals will concentrate their foraging activity around fruiting trees when available, further indicating that fruit is a favored resource that influences female reproduction (Emmons 2000). I examine the behavioral mating system of large treeshrews in relation to fruit availability, and then use molecular markers to examine the genetic mating system and dispersal patterns of this species.

I studied large treeshrews in both primary and selectively logged rainforests in Sabah, Malaysia, and throughout the dissertation I present comparisons between these two habitats. Southeast Asia has experienced greater rates of deforestation than other tropical regions (Sodhi et al. 2004), and the Malaysian state of Sabah in NE Borneo is typical in that most of its valuable timber has already been extracted (Brookfield et al. 1995). However, 60% of Sabah remains under forest cover because its forests were

selectively logged rather than clear cut (Marsh & Greer 1992). Most vertebrate species are present after selective logging, but population densities and behavior may change dramatically (Grieser Johns 1997). For example, both white-handed gibbons (*Hylobates lar*) and mitred leaf monkeys (*Presbytis melalophos*) reduced their activity levels and day range lengths immediately following logging in peninsular Malaysia, and *P. melalophos* abandoned territorial behavior in favor of mutual avoidance strategies to reduce energetically-costly competition for food (Johns 1986). Two species of mousedeer (*Tragulus javanicus* and *T. napu*) were also less common in logged forest due to reduced abundance of certain small fruits and *Ficus* spp. (Heydon & Bulloh 1997), and exhibited home ranges that were twice as large in logged areas than in the primary forest (Ahmad 1994). Single-species research will not provide a comprehensive picture of the impacts of selective logging on wildlife, but comparative studies will become possible as data accumulate from projects such as this one. Additionally, working in more than one habitat can provide important data on intraspecific variation in mating systems.

The objectives of this study are to 1) examine behavioral monogamy in relation to fruit availability, 2) determine whether behavioral monogamy is associated with sexual and genetic monogamy, and 3) examine the consequences of monogamy for dispersal patterns in large treeshrews. To examine behavioral monogamy, I describe the space use, ranging patterns, and home range sizes of large treeshrews using radiotelemetry data from 46 individuals. I also examine differences in these behaviors in primary and selectively logged forests, and in forest undergoing a mast fruiting using data that were collected by Louise Emmons (2000) at one of my study sites. To examine genetic monogamy, I develop molecular markers and examine whether large treeshrews exhibit

substantial extra-pair paternity. Because copulations and other sexual behaviors could not be observed directly in large treeshrews, I present a comparative analysis of testis size and mating systems in treeshrews and primates to investigate sexual monogamy. To examine the influences of monogamy on dispersal patterns, I use molecular genetic analyses to examine whether dispersal and gene flow are female-biased in large treeshrews.

In Chapter I, I present evidence that large treeshrews exhibit behavioral monogamy across a range of ecological conditions. Space use and ranging patterns indicate that males and females form dispersed pairs that occupy a joint territory, but travel and sleep alone. I introduce the new term *asocial monogamy* to describe this mating system. Asocial monogamy where pair members are dispersed in space is predicted to lead to high rates of extra-pair paternity, in contrast to associated pairs that spend most of their time in close proximity (van Schaik & Kappeler 2003). I also find higher amounts of fruitfall in a selectively logged vs. primary forest, and show that treeshrews in the logged forest exhibit better body condition than individuals in primary forest. I reanalyze radiotelemetry data from Emmons (2000) to show that males traveled longer distances and females exhibited better body condition during a mast fruiting than during non-masting periods at the same site. These results support the intraspecific foraging competition and/or predation hypotheses for the evolution of behavioral monogamy.

In Chapter II, I use molecular markers to investigate the genetic mating system of large treeshrews. I report one of the highest rates of extra-pair paternity ever recorded for a behaviorally monogamous mammal, as predicted for mammals that form dispersed

pairs. The analysis also provides evidence of multiple paternity in three litters. These results are consistent with the prediction that asocial monogamy renders mate guarding ineffective (Schülke & Ostner 2005). However, I also find that extra-pair paternity is not associated with large testis size, thus suggesting that intense sperm competition is not an important outcome of the *T. tana* mating system. Comparative analyses using phylogenetically independent contrasts indicate that the testes of treeshrew and primate taxa with uni-male mating systems (monogamy or polygyny) are consistently smaller than the testes of primates with multi-male mating systems. I also discuss previously unappreciated sociobiological similarities between treeshrews and nocturnal prosimians that have important implications for the reconstruction of ancestral primate behavior.

In Chapter III, I use multiple genetic analyses to examine dispersal and gene flow in large treeshrews. Female-biased dispersal is predicted to occur in monogamous species when females intensely compete for ecological resources, but the hypothesis has rarely been tested in mammals (Greenwood 1980; Dobson 1982). First, I use analyses of multilocus microsatellite genotypes to show that females have lower population assignment indices and lower pairwise relatedness values than males, as predicted if the local female population contains a higher proportion of immigrants than the male population. Second, I calculate coalescent-based Bayesian estimates of migration rates between the primary and logged forest populations using mitochondrial DNA and microsatellite markers. Comparison of the two estimates shows that the effective number of female migrants is more than three times the number of male migrants. These results provide the strongest genetic support to date for the predicted association between monogamy and female-biased dispersal in mammals. I suggest that competition between

females for feeding territories creates a sexual asymmetry in the costs and benefits of dispersal in large treeshrews, and that these costs and benefits are closely linked to monogamy in *T. tana*.

FIGURE LEGENDS

Figure 1. The large treeshrew, *Tupaia tana*, a) photographed in the hand, and b) digitally illustrated by natural history illustrator Carl Dennis Buell. Relatively large body size, a long muzzle, reddish pelage, black dorsal stripe, and light shoulder stripe distinguish the large treeshrew from other *Tupaia* species (pgs. 13-14).

FIGURES

a)

b)

CHAPTER I

Behavioral monogamy and fruit availability in the large treeshrew (*Tupaia tana*)

ABSTRACT

Behavioral monogamy in mammals varies from male-female pairs that spend most of their time in close spatial contact (associated pair-living) to pairs that occupy exclusive territories but travel, forage, and sleep alone (dispersed pair-living). I present radiotelemetry data on 46 adult large treeshrews (*Tupaia tana*) from two populations in Sabah, Malaysia that indicate that this species forms dispersed pairs across a range of ecological conditions. Dispersed pair-living was the primary social organization and behavioral mating system in primary forest during a major fruit masting event, in non-masting primary forest, and in selectively logged forest with relatively abundant fruitfall. Behavioral partners were less spatially concordant than partners of other species that form dispersed pairs, and both male and female territories typically overlapped the boundaries of one to three extra-pair territories. Asocial monogamy is presented as a new term to describe the behavioral mating system of this species. Comparison between masting and non-masting forests indicated that females exhibited better body condition during masting, whereas males exhibited larger home range areas and longer daily movements. Both males and females exhibited better body condition in selectively logged vs. primary forests, but ranging patterns were not significantly different between

these habitats. I argue that predation and/or intraspecific foraging competition are the most likely explanations for the evolution of dispersed pair-living in *T. tana*.

INTRODUCTION

Monogamous mating systems occur in only five percent of mammalian species overall (Kleiman 1977; Clutton-Brock 1989), but are much more prevalent among the Euarchonta (15%; dermopterans, treeshrews, and primates), canids, rodents, and some nocturnal taxa, such as dwarf lemurs (Fietz 1999) and bats (McCracken & Wilkinson 2000). Characterizing monogamy has been aided by recent molecular genetic studies that have failed to confirm exclusive mating in behaviorally monogamous species (e.g. Fietz et al. 2000; Goossens et al. 1998; Spencer et al. 1998; Schülke et al. 2004). These results underscore the need to understand the ecological and social factors promoting behavioral monogamy, or pair-living, as a phenomenon distinguished from genetic monogamy in mammals (Reichard 2003).

Early hypotheses for the evolution of behavioral monogamy stressed the importance of biparental care to reproductive success (obligate, or Type I, monogamy, Kleiman 1977; Clutton-Brock 1989; e.g. California mouse, *Peromyscus californicus*, Gubernick et al. 1993; Djungarian hamster, *Phodopus campbelli*, Wynne-Edwards 1987; American beaver, *Castor canadensis*, Sun 2003). However, biparental care evolved secondarily in most pair-living mammalian lineages (Komers & Brotherton 1997), and these hypotheses cannot explain behavioral monogamy in mammals without substantial paternal care. Ecological scenarios argue that high spatial dispersion of females promotes pair-living by preventing males from monopolizing more than one female (Emlen & Oring 1977; e.g. golden-rumped elephant shrew, *Rhynchocyon chrysopygus*,

FitzGibbon 1997), or that intensive mate guarding strategies arise when female home ranges are small, exclusive and defensible (e.g. Kirk's dik-dik, *Madoqua kirkii*, Brotherton & Komers 2003). A third group of behavioral hypotheses predict that males gain enhanced fitness from pair-living by providing services that increase female survival or reproduction. These services may include protection from predation (van Schaik & Dunbar 1990; Kleiman & Malcolm 1981; trail maintenance in long-eared elephant shrews, *Elephantulus rufescens*, Rathbun 1979), protection from infanticide (Kappeler & van Schaik 2002; especially in primates, van Schaik & Kappeler 2003) and other forms of male aggression (Smuts & Smuts 1993), or foraging competition (Wittenberger & Tilson 1980).

A single ecological factor is unlikely to explain behavioral monogamy in mammals, because monogamy occurs in diverse forms across taxa. Behavioral monogamy in pair-living species may vary from pairs that remain cohesive in space and time (associated pairs) on a common territory (*Peromyscus* spp., Ribble 2003), to pairs that may share a territory but travel, forage and sleep alone (cape porcupine, *Hystrix africaeaustralis*, Corbet & Van Aarde 1996; maned wolf, *Chrysocyon brachyurus*, Dietz 1984; elephant shrews, *E. rufescens* and *R. chrysopygus*, Rathbun 1979; Zanzibar galago, *Galagoides zanzibaricus*, Harcourt & Nash 1986; dispersed pairs in primates, Kappeler & van Schaik 2002; van Schaik & Kappeler 2003). Many nocturnal prosimians form dispersed pairs and deserve special consideration because they may reflect the ancestral primate condition (Müller & Thalmann 2000). Dispersed pair-living in a few of these species has been described in detail (fat-tailed dwarf lemur, *Cheirogaleus medius*, Fietz 1999; fork-marked lemur, *Phaner furcifer*, Schülke & Kappeler 2003), and may have

evolved due to fitness gains to females from reduced competition for scarce, patchily-distributed food resources (Schülke & Ostner 2005).

In this study, I examine behavioral monogamy in the large treeshrew, *Tupaia tana* (Mammalia, Scandentia), in Sabah, Malaysia. Male-female pairs in *T. tana* and a few other tupaiids live on joint territories, but forage solitarily and never share sleeping sites (*T. gracilis*, *T. longipes*, and *T. tana* in Borneo, Emmons 2000; previously described as "solitary ranging pairs" in *T. glis* in Singapore, Kawamichi & Kawamichi 1979). Here I present the most detailed study of pair-living in treeshrews to date, using radiotelemetry data from 22 adult *T. tana* in lowland primary rainforest in Sabah, Malaysia (NE Borneo). I also use spatial data collected from 17 adults during a fruit masting episode and seven adults in selectively logged forest to investigate the influence of short- and long-term changes in fruit abundance on behavioral monogamy, respectively. I examine variation in space use and ranging patterns of *T. tana* in these different habitats, and then evaluate alternative evolutionary hypotheses for pair-living in this species. In particular, I calculate two indices of territorial defendability (Mitani & Rodman 1979; Lowen & Dunbar 1994) to examine Emlen and Oring's (1977) prediction that monogamous males are unable to effectively monopolize more than one female's home range.

METHODS

Study Sites

I studied large treeshrews in primary lowland rainforest in Sabah, Malaysia from August to December 2002-2004. This study also includes a reanalysis of radiotelemetry data collected by Emmons (2000) during a major fruit masting event from September to

December 1990, and after the mast from March to September 1991. Both studies were conducted in forest that is part of the Danum Valley Conservation Area (Danum, 4°58´N, 117°48´E). Danum represents the largest lowland rainforest in Borneo likely to remain undisturbed indefinitely (438 km^2), and is nested within a much larger timber concession that comprises nearly 13% of the entire land area of Sabah (Marsh & Greer 1992). Most of the concession surrounding Danum was selectively logged in the 1980's and then left to recover without subsequent disturbance.

Climate and phenology at Danum do not follow strongly predictable patterns, but September through January tends to have the highest recorded rainfall and fruit abundance (Walsh & Newbery 1999). Community-wide synchronous reproduction of trees in the family Dipterocarpaceae, known as mast fruiting, occurs every 5-13 years in Borneo (Janzen 1974; Curran & Leighton 2000). Emmons (2000) observed that the reproductive output of large treeshrews was two to three times higher than normal during the 1990 fruit mast in Sabah, presumably due to increased resources for reproduction. I chose August to December for the study periods in 2002-2004 because *T. tana* reproduction approximately corresponds to periods when fruit abundance is highest (Emmons 2000), and I wished to maximize sampling of juveniles to describe the genetic mating system of this species.

I also studied large treeshrews in selectively logged forest from September to December 2003-2004 within the Malua Forest Reserve (Malua, 5°5´N, 117°38´E), approximately 53 km from the primary forest site. Malua was logged in the early 1980s and has yet to recover the multiple closed canopies (typically 10 m and 20-30 m in height) and tall emergent trees (up to 70 m) that characterize lowland dipterocarp

rainforests (Whitmore 1984). This site is limited to a 10 m canopy composed largely of pioneer tree species, particularly *Macaranga* spp., and is representative of logged forests throughout Sabah (G. Reynolds, personal comment). Selective logging may increase fruit abundance if surviving trees and subsequent pioneers exhibit increased reproductive activity due to greater solar input (Johns 1988). Previous studies in peninsular Malaysia and Borneo have recorded either higher (Chivers 1972; Laidlaw 1994; Hussin 1994) or no overall differences (Heydon & Bulloh 1997) in fruit production after selective logging. This site was replanted with mixtures of dipterocarp seedlings in 2003-2004 for the Sabah Biodiversity Experiment, a large-scale effort to investigate the influence of dipterocarp diversity on ecosystem functions (Schilthuizen 2003).

Study Species

The large treeshrew is a small (200-250 g), diurnal frugivore-insectivore that inhabits the lowland rainforests of Borneo and Sumatra. I chose to study behavioral monogamy in the large treeshrew because previous studies established that *T. tana* is one of the most common rainforest mammals in Sabah (44-54 individuals / km^2), and forms male-female pairs with approximately concordant territorial boundaries (Emmons 2000). Furthermore, several aspects of large treeshrew biology indicate that this species may respond behaviorally to variation in fruit abundance. *T. tana* females have a litter size of two, and typically give birth one to three times a year. However, they exhibit postpartum estrus and are capable of reproducing nine times annually in captivity if fed fruit *ad libitum* (Emmons 2000). Females also exhibit a unique, energetically-costly maternal care system, whereby females deposit their young in a secluded nest that they

subsequently visit only once every 48 hours for intense bouts of nursing (Martin 1966). Both *T. tana* males and females are extremely active and spend almost their entire activity period foraging. Although primarily insectivorous, *T. tana* individuals will concentrate their foraging activity around fruiting trees when available, suggesting that fruit is a favored resource that influences reproduction (Emmons 2000).

Data Collection

The same trapping transects and similar general methodology were used in 2002-2004 as Emmons (2000) employed at Danum in 1990-1991. Salient differences between the earlier study and the recent data collection methods are noted below. Large treeshrews were trapped at each site with locally-made wire mesh traps placed every 25 m along two 500 m transects in 1990-1991, and three transects in 2003-2004. In 2004 I placed two additional 500 m transects at the logged site to increase captures. I conducted trapping sessions every 3-4 weeks during the study period, and habituated animals by pre-baiting open traps for two days before each session. Traps were baited and set at 0600 h with slices of a local variety of banana (local name: *pisang emas*) previously established as optimal for capturing tupaiids (Bernard 2003), and checked twice daily at 1030 h and 1500 h. Captured animals were transferred to cloth bags, weighed, and sedated with a ketamine hydrochloride injection (10 mg / kg dose). Treeshrews were marked with ear tags and tail hair clipping in 1990-1991. During the 2002-2004 study period, I measured hind foot length, collected hair samples and ear clips for genetic analyses, and injected animals with a subdermal passive integrated transponder (Biomark, Inc., Boise, ID) for

permanent identification. I noted lactation and checked for the presence of embryos through palpation of female abdomens.

If adults were in good condition, then I fitted them with radio collars manufactured by Wildlife Materials Inc (1990-1991, 2002, model SOM-2190, ~4.5-5.0 g) or Holohil Systems Ltd (2003-2004, model PD-2C, ~4.0 g). Juveniles were identified by their small size (mass < 180 g, based on growth curve in Emmons 2000) and the presence of milk teeth or newly-erupted unworn adult teeth, and were not collared unless trapped later as adults. To avoid confounding effects of age, I excluded juveniles that were radio-tracked in the same study period as their birth from analyses of space use in adult *T. tana*. Animals were released at the site where they were captured after recovering from sedation (two to three hours). This study includes radiotelemetry data from four adult males and five females in primary forest in 1990, four males and four females in 1991, three males and three females in 2002, four males and three females in 2003, and five males and four females in 2004. In logged forest I tracked three males and one female in 2003 and two males and three females in 2004. Collar failure rates for 1990-1991 are reported in Emmons (2000). I switched to a different collar manufacturer after a failure rate of 40% in 2002, resulting in only one collar failure in 2003 and 2004 combined.

I measured and marked the trail system at each study site at 25 m intervals to facilitate localization of telemetry signals. Radio-collared treeshrews were followed throughout their entire activity period (0600 h until nesting at 1530 to 1800 h) on foot by an observer with a radio receiver for three consecutive days to estimate home range sizes and day range lengths. Compass bearings in the direction of the animal's radio signal

were taken every 20 min from three different marked sites. If three different compass bearings could not be taken within five minutes, the tracker started the readings over. Emmons (2000) established that *T. tana* home range areas no longer increased after collecting more than three days of location points. Nevertheless, three weeks or more after the original three-day tracking period I followed most collared individuals for one or two additional days. Simple linear regression of 95% kernel home range area in hectares (ha; see below for calculation) on the number of locations recorded per individual indicated that our estimates of home range size did not increase with these additional tracking points ($y = 4.45 \pm 0.002x$; $N = 46$, $P = 0.85$, $R^2 = 0.001$). Additionally, all animals that were located during the additional tracking days remained on the same ranges recorded during the initial three-day session. This study includes a total of 1,562 hours of radiotelemetry observations on 46 adult *T. tana* in masting forest in 1990 ($N = 8$ adults, 322.5 hrs), post-masting forest in 1991 ($N = 9$, 312.9 hrs), primary forest in 2002-2004 ($N = 21$, 679.9 hrs), and selectively logged forest in 2003-2004 ($N = 8$, 247.1 hrs). I also radio-tracked 10 sub-adult *T. tana* for a total of 328 hrs during the 2002-2004 study periods.

I tracked one focal individual at a time instead of a behavioral pair because male-female partners could not always be trapped and collared at the same time. Additionally, signal attenuation caused by dense tropical vegetation made it difficult and inefficient to track two individuals foraging alone. However, several times during a tracking day I simultaneously checked the radio signals from other collared individuals on the study site, and noted when they were in proximity to the focal individual's signal. I also noted visual sightings of the focal individual and any other treeshrews nearby.

In 2003 and 2004 I used fruit traps to compare fruitfall between the primary and logged sites. I used fruit traps because only fruit that has fallen to the ground is available to large treeshrews (Chapman & Wrangham 1994). The traps consisted of a one m^2 section of plastic netting suspended 60 cm above the ground by four PVC pipes, and were installed every 50 m along the trapping transects ($N = 41$ fruitfall traps at each site). I collected the entire contents of each trap every week and sorted the soft, fleshy fruits from other materials. I recorded the number of individual fruits and total wet weight in grams (g) for each trap, and then dried the fruits in an 80° C oven before recording the dry weight (g). Fruitfall is not reported for 2004 because the fruit traps in logged forest were repeatedly destroyed by elephants.

Radiotelemetry and Spatial Analyses

I triangulated radiotelemetry bearings and calculated error polygons for each individual tracked from 2002-2004 using the Lenth maximum likelihood estimator in the software program Locate II (Nams 2000), and then imported the location points and error ellipses into ArcView GIS 3.3 (ESRI 2002). Location points for radio-collared treeshrews tracked by Emmons (2000) in 1990-1991 were digitized from hand-drawn maps using the software program WinDIG 2.5 (Lovy 1996), imported into ArcView, and then analyzed using the methods described below. For each individual, I calculated 95% minimum convex polygon home ranges (MCP; 5% of outlying observations excluded using harmonic mean method), 95% kernel home ranges (fixed kernel; smoothing parameter chosen using least squares cross validation) and minimum day range length using the Animal Movement extension (Hooge et al. 1999) in ArcView. I calculated MCP home

ranges for comparison with studies on other taxa, but kernel home ranges were used for statistical analyses because kernel methods are very robust to autocorrelation and do not constrain the geometry of territorial boundaries as severely as MCP (Kernohan et al. 2001). I measured minimum day range length as the cumulative linear distance between sequential locations recorded for an individual in a single day.

Having described the location and shape of adult territories for each site in each study period, I designated behavioral pairs of *T. tana* when at least 50% of a female's territory was contained within the territory of a single male. Spatial concordance between male-female pairs was quantified as the percentage overlap between their 95% kernel home ranges using the "Clip by shape" function of the Home Range extension (Rodgers & Carr 1998) in ArcView. The number of opposite-sex extra-pair ranges overlapping each individual's 95% kernel home range during a given study period was recorded, and the percentage overlap with both same- and opposite-sex extra-pair individuals, were calculated using the methods described above.

Statistical Analyses

I examined sexual dimorphism in body mass, 95% kernel home range size, and mean day range length using data from individuals captured in primary forest from 2002-2004 to avoid influences of fruit masting and selective logging. I tested for sex differences using two-sample *t*-tests assuming unequal variances. Parametric correlation analysis was used to examine whether 95% MCP and kernel analyses produced similar estimates of home range size.

To examine overall fruit production and phenology in primary vs. logged forests, I calculated mean ± standard errors in weekly dry weight of fruit (g / trap) collected by fruitfall traps in 2003 and tested for an overall difference between sites using a matched pairs *t*-test. I compared the relative condition of treeshrews in different ecological conditions by using the residuals of a least-squares regression of body mass (g) on hind foot size (mm). Residual body mass was used to examine differences in mass due to factors other than overall skeletal size. Body mass values for females known to be pregnant were excluded from these analyses.

To examine pair-living in relation to short term increases in fruit abundance, I compared body condition, home range area, mean day range length, and number of overlapping extra-pair territories between individuals in primary forest during the 1990 masting, primary forest after masting in 1991, and primary forest from 2002-2004. The post-masting forest in 1991 was considered separately because population turnover through either death or displacement of former residents led to a new group of individuals on the study site after the mast (Emmons 2000). I compared the same variables between treeshrews in primary forest from 2002-2004 and selectively logged forest from 2003-2004 to examine pair-living in relation to long-term changes in fruit abundance. In both cases I tested for significant differences in the variable of interest using a two-way ANOVA with sex, forest category, and the interaction between sex and forest category as model effects. I excluded the interaction between sex and forest category when it did not contribute to the overall significance of the model. I used 10,000 replicates of randomized unbalanced factorial ANOVA (Manly 1991) with sex, forest category, and the interaction term as model effects to examine differences in percent territorial overlap

with behavioral partners and both same- and opposite-sex extra-pair individuals. I used randomization techniques because these overlap percentages were measured for male-female dyads and thus are not independent observations.

I used analysis of covariance to examine the influence of day range length, sex, and the interaction between day range length and sex, on home range size because males and females may use their ranges to control access to different resources. Finally, I calculated two indices of territorial defendability for males in masting forest in 1990, post-masting forest in 1991, primary forest in 2002-2004, and selectively logged forest in 2003-2004 to examine whether pair-living in *T. tana* can be explained by the dispersion of females. These two measures, *D* (Mitani & Rodman 1979) and *M* (Lowen & Dunbar 1994), are based on the relationship between day range length and territory size in territorial and non-territorial primate species. I used SAS ver. 8.02 (SAS Institute 2001) for the randomization tests and JMP ver. 5.0.1.2 (SAS Institute 2003) for all other statistical analyses.

RESULTS

Space use and ranging patterns of large treeshrews

T. tana in primary forest from 2002-2004 did not exhibit significant sexual dimorphism in body mass ($N = 7$ females and 12 males, $t = 0.41$, $P = 0.69$), territory size ($N = 9$ females and 13 males, $t = -1.29$, $P = 0.21$), or mean daily distances traveled ($N = 9$ females and 13 males, $t = -0.16$, $P = 0.88$). Home range analyses indicated that male-female pairs of large treeshrews occupied joint areas in all habitats examined in this study (Figures 1-4). Spatial coordination between male and female ranges estimated using 95%

MCP and kernel estimates was qualitatively similar (Figures 1-4), and these two analyses produced highly correlated estimates of home range area ($N = 46$, $r = 0.92$, $P < 0.0001$). The location of individuals' home ranges did not change within study periods, but only two adults survived for two entire study periods (F14 in primary forest and M35 in selectively logged forest in 2003-2004). Two males disappeared within the first month of the 2003 study period in primary forest (M14 and M20) and were quickly replaced by new males that occupied similar home ranges (M28 and M29, respectively).

The percentage of individuals' 95% kernel home range that overlapped with their partner was highly variable across sites, averaging from 36-62% for males and 62-72% for females (Table 1). Overlap with opposite-sex extra-pair individuals was common for both males and females, but averaged only 7-20% of home range area (Figures 1-4, Table 1). No male ranges overlapped more than 50% of two separate female ranges. Mean home range area varied from 3.4-4.2 ha for females and 4.0-6.9 ha for males, and individuals traveled over one km per day within their home ranges regardless of sex or study period (Table 1).

Direct sightings of radio-collared treeshrews in the 2002-2004 study periods were rare ($N = 20$) due to the dense vegetation and my desire to avoid disturbing individuals' normal behavior by pursuing them off-trail. All direct sightings were of solitary, foraging individuals. While radio-tracking a focal individual, I also monitored the location of other collared treeshrews throughout the day. The direction and strength of radio signals indicated partners occasionally spent more than one tracking interval (≥ 20 min) in close proximity ($N = 7$), and in one of these cases spent two entire days together (F14-M19 pair in 2003). Other cases of proximity ($N = 3$) involved a radio-collared sub-

adult and adult on the same territory. During the 2004 study period in primary forest, I observed an adult (M40) male and sub-adult (m37) male on adjacent territories engaging in chasing and calling at the common boundary of their respective territories. Three subsequent tracking days indicated that m37 did not enter the area of dispute again (Figure 1).

Sub-adults were either spatially associated with an adult pair, or used relatively small, exclusive ranges (Figure 1). I suspected two instances of predation in 2004 when I recovered the damaged, hair-covered radio-collar of a sub-adult female in 2004 (f23), and a sub-adult male's (m37) radio-collar buried under six inches of leaf litter and soil. One radio-collared adult male (M19) in 2003 was eaten by a mangrove snake (*Boiga dendrophila*, Munshi-South 2005). Other radio-collared sub-adults either disappeared due to unknown causes ($N = 2$) or remained on the site for the duration of the study period ($N = 6$).

Space use and ranging patterns in relation to fruit masting

Males in primary forests exhibited significantly larger home ranges and longer day range lengths than females, and day ranges were significantly longer during the masting period (Table 2, Figures 1-3). Female home ranges overlapped their male partner's ranges to a greater degree than male ranges overlapped female partners' ranges (Table 2): an overall average of 70% of a female's home range was contained within the range of a single male, whereas this value was only 48% for males. No differences were found in either the number or percentage area of overlapping extra-pair ranges. Body condition varied widely among the masting, post-masting, and non-masting study periods in primary

forest (Table 1), but female body condition was superior to male body condition during the masting. The significant interaction between sex and forest type reflected higher values for female body condition during the masting period than female body condition values in non-masting years (Table 2).

Space use and ranging patterns in relation to logging

As above, females exhibited greater home range overlap with their mate than males (Table 3). Of the spatial and behavioral factors compared between primary and logged forest in the 2002-2004 study periods, only the number of extra-pair overlapping ranges was significantly different between primary and logged forests (Table 3). The mean number of overlapping extra-pair territories was nearly three times fewer in logged forest than in primary forest for both sexes (Tables 1, 3). Body condition values were also significantly higher in logged forest than in primary forest (Table 3). The temporal pattern of fruitfall in the logged and primary forest sites was similar during the study period in 2003, but the weekly mean dry weight of fruit per trap was consistently greater in logged forest (Figure 5; weekly mean fruit per trap in primary forest = 0.09 ± 0.07 g, logged forest = 0.54 ± 0.08 g, $t = 4.16$, $P = 0.006$).

Home range size, day range length, and defendability indices

Kernel home range size increased with mean day range length (Figure 6; $F_{3,42} = 20.6$, $P < 0.0001$, $R^2 = 0.41$) but there was no effect of sex ($F = 1.8$, $P = 0.19$) or the interaction between day range length and sex ($F = 1.6$, $P = 0.22$). I calculated two indices of territorial defendability that measure the ability of an animal to monitor the boundaries of

its home range. The Mitani-Rodman index (*D*) values calculated for male *T. tana* during each study period were substantially higher than the cutoff value calculated for territorial vs. non-territorial primates (Table 4, $D \geq 0.98$ for territorial primates, Mitani & Rodman 1979). Values ranged from 5.32 for the 1991 post-masting period to 6.34 for selectively-logged forest in 2003-2004. I calculated the Lowen-Dunbar index of defendability (*M*) assuming a mean intruder detection distance of 50 m or 10 m for male *T. tana*, and these values also greatly exceeded the cutoff for territorial primates (Table 4, $M \geq 0.08$ for territorial primates, Lowen & Dunbar 1994). The latter conservative detection distance assumption resulted in *M* values ranging from 0.2 in masting forest to 0.25 in selectively logged forest.

DISCUSSION

Behavioral mating system of large treeshrews

Male-female pairs of large treeshrews occupied joint, spatially-associated home ranges (Figures 1-4). A significantly higher percentage of the female partner's range was typically contained within the male's home range, but no males were associated with two female home ranges despite this sex difference in spatial cohesion. The consistency of pair-living across study periods indicates that *T. tana* exhibits "uniform" (> 90% of social groups are pairs) rather than "variable" pair-living (van Schaik & Kappeler 2003). Most individual home ranges slightly overlapped the spatial boundaries of opposite- and same-sex ranges, but individuals were much less spatially associated with extra-pair individuals than with their partner. Pairs occupied the same home ranges throughout the four-month

study periods, which is long enough for two to three reproductive events. However, pairs did not persist for more than one year, presumably due to mortality or migration.

Formation of two-adult groups does not necessarily imply pair-bonding (e.g. primates, Fuentes 2002), belying the need to distinguish between associated and dispersed pairs *sensu* van Schaik and Kappeler (2003). Due to the elusive nature and nearly constant activity of *T. tana*, it was impossible to assess quantitatively affiliative behaviors indicative of pair-bonding, such as patterns of proximity or reciprocity between pair members (Fuentes 2002). However, all of our direct observations were of solitary individuals, and radiotelemetry indicated that treeshrews were rarely in proximity to other individuals. Emmons (2000) also predominantly observed solitary *T. tana*. These results indicate that *T. tana* form dispersed pairs, and exhibit less spatial and behavioral cohesion than dispersed pairs in closely-related primates (Fietz 1999; Schülke & Kappeler 2003).

The phrase "asocial monogamy" may best characterize the behavioral mating system of large treeshrews, as the lack of social interactions among large treeshrew individuals starkly contrasts with the "social monogamy" often observed and described in mammals and birds where pairs are in frequent contact and exhibit affiliative behaviors (e.g. multiple studies in Reichard & Boesch 2003)(Gowaty 1996). Only recently have comparative studies indicated that the dispersed pair-living form of behavioral monogamy (asocial monogamy) may have arisen through a different evolutionary route (from solitary ancestors) than associated pair-living (from gregarious ancestors, Kappeler & van Schaik 2002; Müller & Thalmann 2000; Brotherton & Komers 2003). The basal treeshrew species, the pentail (*Ptilocercus lowii*), exhibits associated pair-living (Emmons 2000), and other *Tupaia* spp. engage in substantial pair bonding behavior (e.g.

T. belangeri, Martin 1968). However, reconstruction of the ancestral mating system of *T. tana* and other *Tupaia* spp. requires information on the mating systems of poorly-studied intermediate taxa between *P. lowii* and *T. tana* (particularly *Dendrogale* spp., Olson 2005).

Space use, ranging patterns, and fruit abundance

Large treeshrews formed dispersed pairs in all ecological conditions examined in this study, but differences in home range use and overlap were observed between study periods. Comparison of males and females during masting and non-masting periods indicated that males exhibited substantially larger territories, longer day ranges, and less territorial overlap with their behavioral partners during the masting period (Figures 2 and 3). Only female body condition increased in response to the masting. Some females gave birth to three litters in succession, indicating the potential for rapid reproductive response of large treeshrews to increases in fruit abundance (Emmons 2000). During similar time spans in non-masting primary forest in 2002-2004, females gave birth to zero or one litter, with only one female reproducing twice in succession (J. Munshi-South, unpublished data). Females may have used the extra resources provided by fruit masting for increased reproduction, whereas males may not have exhibited substantial weight gain because they used the extra resources for increased daily movements. Despite increased ranging, males did not gain greater overall access to female territories during the masting period.

I recorded consistently higher fruitfall and better treeshrew body condition in logged forest than in primary forest. I cannot definitively rule out other explanations for

superior body condition, but this result in conjunction with the pattern found during the mast fruiting implicate fruit abundance as an important causal factor. The availability of invertebrate prey could have been greater in the selectively logged forest, but a previous study at Danum found that litter invertebrates were less abundant in logged forest than in primary forest (Burghouts et al. 1992). Fewer species, but not overall abundance, of moths (Willott 1999) and termites (Eggleton et al. 1999) were also recorded in logged forest than in the primary forest at Danum.

I did not find the same differences in space use in logged forest that I found in masting forest. It is unclear why female home ranges were not smaller in logged forest, but the population density of competitors may have been lower despite greater fruit abundance and better body condition. Logging in southeast Asia often results in a greater frequency of large treefall gaps than in primary forest (Whitmore 1984; Grieser Johns 1997), and the spatial pattern of treeshrews in logged forest indicated that pairs occupied islands of suitable habitat that were separated by gaps of unoccupied, sub-optimal habitat (Figures 1, 4). Individuals occasionally entered and moved across treefall gaps but did not engage in any sustained activity within them (J. Munshi-South, personal observation), suggesting that forest structure is a more important influence on treeshrew space use than fruit abundance in logged forest. Alternatively, I may not have sampled adults on the logged site that were using treefall gaps as home ranges.

Evaluation of hypotheses for the evolution of pair-living in *T. tana*

Several hypotheses have been proposed for the evolution of pair-living, but no single hypothesis has been robustly supported in mammals. Recent reviews of pair-living in

primates found support for contrasting sets of explanations: in one case, energetic constraints, predation reduction, and mate guarding (Fuentes 2002), and in the other, infanticide reduction and predation reduction through nest-guarding (van Schaik & Kappeler 2003). Below I discuss the relative support for different evolutionary hypotheses for pair-living in *T. tana*, and throughout refer to predictions previously developed for pair-living mammals (many of these predictions were originally developed for primates due to intensive focus on that group; e.g. see Table 1 in Fuentes 2002; van Schaik & Kappeler 2003).

Does female dispersion explain pair-living in large treeshrews?

Emlen and Oring (1977) predicted that pair-living will occur when females are so widely dispersed that males cannot monopolize more than one reproductive female. For example, when male elephant shrews (*R. chrysopygus*) defend two female territories, they experience increased activity levels, weight loss, and increased rates of intrusion by neighboring males (FitzGibbon 1997). To examine male defendability of multiple female home ranges in *T. tana*, I used male day range length and home range size to calculate two indices of territorial defendability. The first index, *D*, is successful at predicting territorial defense in primate species using only the ratio of day range length to home range diameter. Territorial primates almost invariably exhibit values of *D* exceeding 0.98 (i.e. species that can travel across their territory in one day, Mitani & Rodman 1979). I calculated *D* values for large treeshrews that were more than five times higher than the cutoff value for primates, indicating that male *T. tana* can routinely cross their territories multiple times in one day.

The *D* index does not account for the length of the territorial boundary that must be defended, so I also calculated a second index of defendability, *M*, that describes the collision rate per unit boundary length (Lowen & Dunbar 1994). Territorial primates exhibit *M* values exceeding 0.08, and again I calculated index values for *T. tana* that exceeded the primate cutoff (Table 4). Assuming the lowest *M* value I calculated for *T. tana*, males exceed the defendability threshold for primates only if they attempt to defend more than three female home ranges. Thus, spatial dispersion of females alone does not explain pair-living in *T. tana*, unless space use of male treeshrews is substantially different from primate space use.

Do male large treeshrews provide services to females?

The largest group of hypotheses for behavioral monogamy propose that pair-living evolved because male partners provide services that enhance the survival and reproduction of their female partners. Many of these hypotheses are unlikely to apply to *T. tana* because absentee maternal care and dispersed pair-living limit male-female and parent-offspring interactions. For example, direct paternal care cannot explain pair-living in large treeshrews because only females visit young in the nest, and care of *T. tana* pups by males has not been recorded in the field or laboratory (Emmons 2000; Martin 1966).

Infanticide prevention appears to be associated with the evolution of pair-living in primates, because males typically protect infants in species that form permanent pairs and carry their young (van Schaik & Kappeler 2003; van Schaik & Kappeler 1997). However, absentee maternal care, female reproductive physiology, and solitary foraging lead us to reject the infanticide prevention hypothesis for *T. tana*. Extra-pair males are

unlikely to know the location of offspring cached in nests visited only briefly by female *T. tana*. In contrast to adults, large treeshrew pups in the nest are nearly odorless and motionless (Emmons 2000). Long lactation periods in relation to gestation make infanticide a successful male strategy in some mammals (van Schaik 2000), but female *T. tana* become receptive to mating almost immediately after giving birth if sufficient food is available (Emmons 2000).

Solitary foraging in *T. tana* also reduces the potential for male defense against predation or sexual harassment. Except in one instance of a pair that spent nearly two entire days together, females in this study were not recorded in close proximity to other individuals. However, I recorded one instance of predation by a snake (Munshi-South 2005) and suspected predation in a few other cases. Diurnal predators such as yellow-throated martens (*Martes flavigula*) and raptors were often observed on the study site, and both male and female *T. tana* sometimes gave alarm calls upon detecting a human observer (J. Munshi-South, personal observation, Emmons 2000). Quantitative assessments of proximity between behavioral partners and male vigilance behaviors are needed before predator defense or protection from male harassment can be ruled out as explanations for pair-living in *T. tana*.

Variations on the protection from male harassment or predation hypotheses predict that males provide indirect protection by maintaining escape routes or shelter sites (Kleiman & Malcolm 1981). For example, males of two elephant shrew species, *R. chrysopygus* and *E. rufescens*, maintain multiple nests or extensive trail systems that are used by females for resting and traveling, respectively (Rathbun 1979). *T. tana* females, however, nest separately from males in hollow logs or trees that have not been obviously

manipulated (Emmons 2000). Large treeshrews avoid open areas and may concentrate their foraging along stream banks or fruit trees (J. Munshi-South, pers. obs., Emmons 2000), but no evidence of trail maintenance has been observed for any treeshrew species (Emmons 2000; Kawamichi & Kawamichi 1979; Martin 1968). Elephant shrews have much smaller home ranges than large treeshrews (0.34 and 1.7 ha for *E. rufescens* and *R. chrysopygus*, respectively, Rathbun 1979), and thus trail and nest maintenance may be a more successful strategy in these species than in *T. tana*.

The final hypothesis in this group proposes that males provide defense against conspecific foraging competition. One version predicts that females pair with a male based on the quality of the feeding territory guarded by that male (Thalmann 2001; Fuentes 2002), whereas another proposes that 1) female-female avoidance due to foraging competition leads to territoriality, and 2) males defend a single female's territory against other males to limit the number of foraging individuals in the same area (intersexual feeding competition hypothesis, Schülke 2005). This two-step scenario has received support from comparative studies of behavioral monogamy in nocturnal prosimians (Müller & Thalmann 2000; van Schaik & Kappeler 2003) and other mammals (Komers & Brotherton 1997).

Several aspects of large treeshrew space use and reproduction suggest that females benefit from reduced foraging competition. Both male and female *T. tana* spend most of their time foraging, resulting in our observations of relatively long daily movements compared to home range areas (e.g. high calculated values for territorial defendability indices, Lowen & Dunbar 1994). Reduced reproductive output in the wild compared to captivity, intense bouts of nursing only once every 48 hours while lactating,

concentrated foraging activity at fruiting trees, and improved female body condition and reproduction when fallen fruit is abundant, indicate that energy limits reproduction in female *T. tana* (this study and Emmons 2000). If females choose male partners based on their feeding territories, some high-quality male territories should support two or more females (variable pairs in van Schaik & Kappeler 2003). The largest male home ranges in this study (> 10 ha) did not support two females, even when fruit was abundant. The observation that males typically defend larger territories than females provides additional support for sex-specific territoriality in *T. tana* and is consistent with the two-step evolution of pair-living described above.

Does male mate guarding explain pair-living?

The mate guarding hypothesis proposes that pair-living evolved because males benefit from monopolizing a single female. Pair-living in mammals is associated with small, exclusive female home ranges, and may represent a risk aversion strategy that guarantees mating with a single female while reducing aggressive encounters with other males (Komers & Brotherton 1997). This hypothesis predicts that males continually monitor their female partner (klipspringer, *Oreotragus oreotragus*, Roberts & Dunbar 2000; gibbons, *Hylobates* spp., van Schaik & Dunbar 1990), and/or infidelity results in costly aggressive conflicts (Kirk's dik-dik, *M. kirkii*, Brotherton & Rhodes 1996). Mate guarding is unlikely to explain pair-living in large treeshrews or other species that forage solitarily and exhibit low spatial cohesion (Schülke & Ostner 2005), unless guarding is intensified while females are receptive (golden lion tamarins, *Leontopithecus rosalia*, Baker et al. 1993; maned wolves, *C. brachyurus*, Dietz 1984). Quantitative data on mate

guarding behavior, such as male over-marking of female scent marks or pair cohesion during receptive periods, will generally be difficult to collect for dispersed pairs of *T. tana*. However, the high rates of extra-pair paternity in *T. tana* revealed by genetic parentage analyses indicate that intensive mate guarding, if it occurs, may not be very successful at assuring paternity in large treeshrews (Chapter 2).

Conclusions

Large treeshrews form monogamous pairs across a range of ecological conditions. However, partners generally travel, forage, and sleep alone, leading me to propose the term "asocial monogamy" to describe this mating system. Male *T. tana* are spatially associated with one female on a joint feeding territory, but generally exhibit larger territories than females and may seek extra-pair mating by extending their territorial boundaries beyond their partner's range. Female treeshrews also typically overlap one to three extra-pair males at the margins of their territory. Reproductive biology and space use indicate that direct male care, infanticide prevention, and female dispersion are not primary explanations for pair-living in large treeshrews. Predation and intraspecific foraging competition may have driven the evolution of pair-living in *T. tana*, but experimental manipulations of resource abundance and predation pressure are needed to determine their relative importance.

TABLES

Table 1 Body mass, space use, and ranging patterns of large treeshrews. Means ± standard error of body mass, 95% kernel home range area, day range lengths, number of opposite-sex, extra-pair overlapping territories, percent of territory overlapping behavioral partner's territory, and percent of territory overlapping both same- and opposite-sex extra-pair (EP) territories are presented for male and female *T. tana* during different study periods (pg. 41).

Study Period	Masting 1990		Post-masting 1991		Primary 2002-2004		Logged 2003-2004	
Variable	Male	Female	Male	Female	Male	Female	Male	Female
Body mass (g)	231 ± 9.2	252 ± 12.1	228 ± 9.0	202 ± 6.6	213 ± 4.9	215 ± 3.5	223 ± 5.1	227 ± 2.9
Territory size (ha)	6.9 ± 1.5	3.4 ± 0.7	4.0 ± 0.1	3.5 ± 0.3	5.5 ± 0.9	4.1 ± 0.7	5.0 ± 1.4	4.2 ± 1.5
Day range length (km)	1.8 ± 0.1	1.2 ± 0.9	1.2 ± 0.07	1.1 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.6 ± 0.3	1.4 ± 0.1
No. extra-pair overlaps	1.5 ± 0.5	1.2 ± 0.4	1.0 ± 0	1.3 ± 0.5	0.8 ± 0.2	1.2 ± 0.3	0.13 ± 0.13	0.3 ± 0.18
% partner overlap	0.36 ± 0.21	0.7 ± 0.15	0.62 ± 0.03	0.72 ± 0.05	0.47 ± 0.05	0.7 ± 0.1	0.49 ± 0.04	0.62 ± 0.02
% EP overlap	0.07 ± 0.02	0.11 ± 0.05	0.14 ± 0.05	0.1 ± 0.05	0.11 ± 0.04	0.2 ± 0.06	0.05 ± 0.05	0.21 ± 0.21
% same-sex overlap	0.12 ± 0.04	0.12 ± 0.07	0.11 ± 0.02	0.12 ± 0.06	0.04 ± 0.02	0.06 ± 0.02	0.02 ± 0.02	0.03 ± 0.02

Table 2 Differences in body condition and space use between large treeshrews in masting and non-masting primary forest. Forest types (FT) include masting (year 1990), post-masting (1991), and non-masting (2002-2004). The degrees of freedom (df) and *F*-values resulting from ANOVAs with sex and forest type as main effects are reported, as well as the R^2 value associated with the entire model. Day range length was $\log_e$-transformed to improve normality. *P*-values for the two percent-overlap variables were computed by comparing the *F*-statistic to a distribution of *F*-statistics computed from 10,000 randomizations of the data. Tests with *P*-values equal to or below a significance level of 0.05 are highlighted in bold and marked with * for $P \leq 0.05$, ** for $P \leq 0.01$, and *** for $P \leq 0.001$ (pg. 42).

Factor	df	Sex	Forest (FT)	Sex * FT	Model R^2
Body Condition	5,30	**3.21***	0.31	**4.33***	0.35
Territory size	3,35	**4.71***	0.65	NS	0.15
Day range length	3,35	**4.50***	**3.79***	2.0	0.31
No. extra-pair overlap	3,33	0.28	0.47	NS	0.04
% pair overlap	3,23	**12.96*****	1.73	NS	0.42
% opposite-sex EP overlap	3,33	0.77	0.41	NS	0.04
% same-sex overlap	3,33	0.18	2.71	NS	0.15

Table 3 Differences in body condition and space use between large treeshrews in primary (years 2002-2004) and logged forest (2003-2004). The degrees of freedom (df) and *F*-values resulting from ANOVAs with sex and forest type as main effects are reported, as well as the R^2 value associated with the entire model. The interaction term between sex and forest type was not significant for any model and thus was not included. Day range length was $\log_e$-transformed to improve normality. *P*-values for the two percent-overlap variables were computed by comparing the *F*-statistic to a distribution of *F*-statistics computed from 10,000 randomizations of the data. Tests with *P*-values equal to or below a significance level of 0.05 are highlighted in bold and marked with * for $P \leq 0.05$ or ** for $P \leq 0.01$ (pg. 43)**.**

Factor	df	Sex	Forest Type	Model R^2
Body Condition	2,21	1.3	**4.69***	0.23
Territory size	2,26	1.55	0.03	0.06
Day range length	2,26	0.24	<0.01	0.01
No. extra-pair overlap	2,26	0.95	**4.92***	0.18
% pair overlap	2,12	**11.24****	0.06	0.48
% opposite-sex EP overlap	2,26	1.46	0.04	0.05
% same-sex overlap	2,24	0.33	0.78	0.04

Table 4. Ranging data and defendability indices for male *T. tana* during four different study periods. I calculated *D* using the formula $d / (4A/\pi)^{0.5}$ in Mitani and Rodman (1979), where *d* equals the average day range length and *A* equals home range area. I calculated *M* using the formula $M = N (sv / d^2)$ in Lowen and Dunbar (1994), where *s* equals the mean intruder detection distance, *v* equals the day range length, and *d* equals $(4A/\pi)^{0.5}$ as defined above. To examine the influence of variable intruder detection distances, I calculated *M* assuming *s* equaled 50 m and 10 m for male *T. tana* (pg. 44).

Study Period	*A* (km^2)	*d* (km)	*D*	*M* (*s* = 0.05 km)	*M* (*s* = 0.01 km)
Masting 1990	0.069	1.8	6.07	1.02	0.2
Post-masting 1991	0.04	1.2	5.32	1.18	0.24
Primary 2002-04	0.055	1.5	5.67	1.07	0.21
Logged 2003-04	0.05	1.6	6.34	1.26	0.25

FIGURE LEGENDS

Figure 1. 95% minimum convex polygon home ranges of behavioral pairs in a) masting forest in 1990, b) post-masting forest in 1991, c) primary forest in 2003, d) primary forest in 2004, and e) selectively logged forest in 2004. Black outlines represent male home ranges, gray outlines represent female home ranges, and solid gray polygons represent sub-adult home ranges. The hatched area in d) represents an area of territorial conflict between an adult and sub-adult male (pg. 47).

Figure 2. a) Male and b) female kernel home ranges in masting forest in 1990, and c) male and d) female kernel home ranges in post-masting forest in 1991. Black areas represent 50% kernel ranges and lighter areas represent 95% kernel ranges (pg. 48).

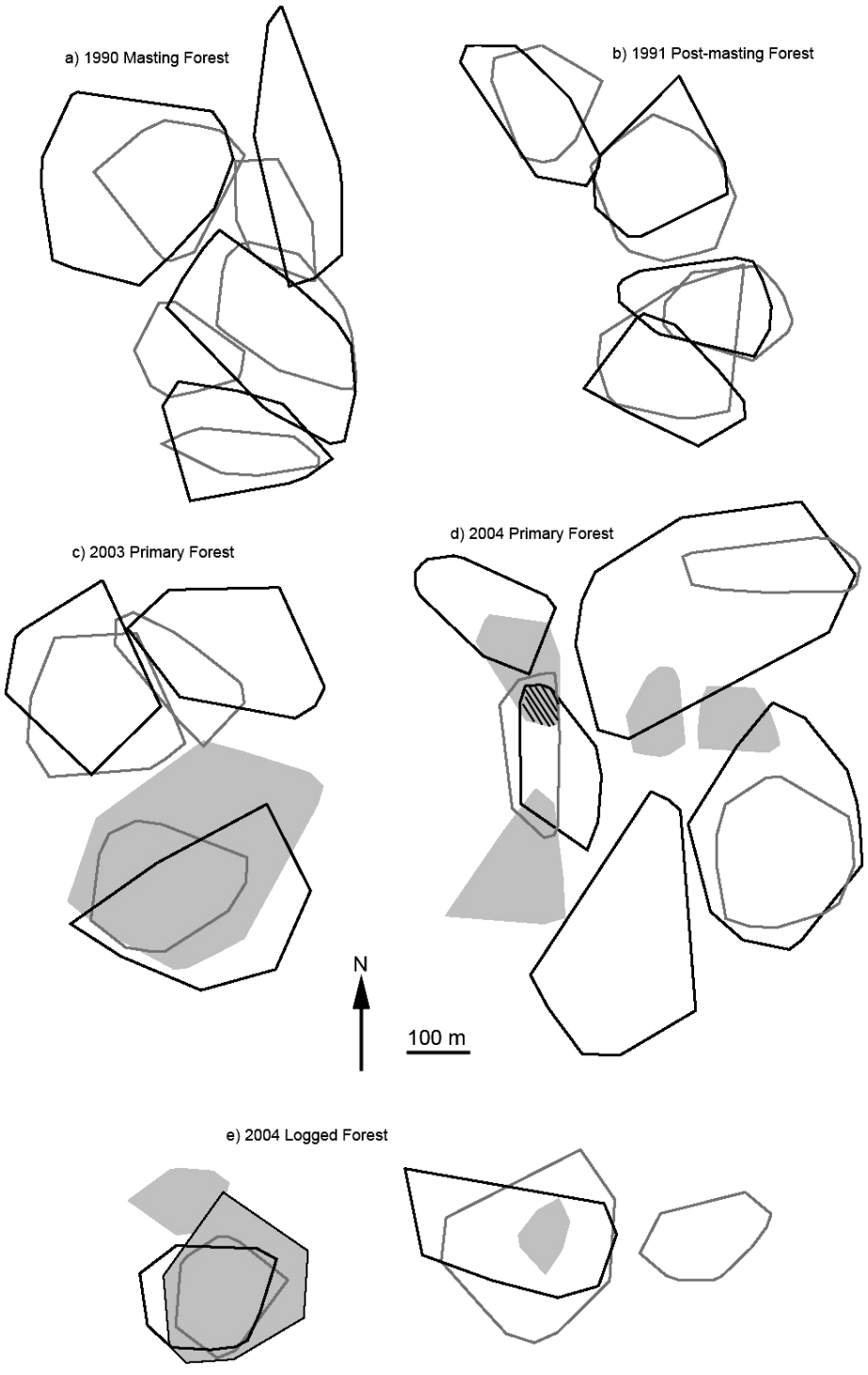
Figure 3. a) Male and b) female kernel home ranges in primary forest in 2002, c) male and d) female kernel home ranges in primary forest in 2003, and e) male and f) female home ranges in primary forest in 2004. Black areas represent 50% kernel ranges and lighter areas represent 95% kernel ranges (pg. 49).

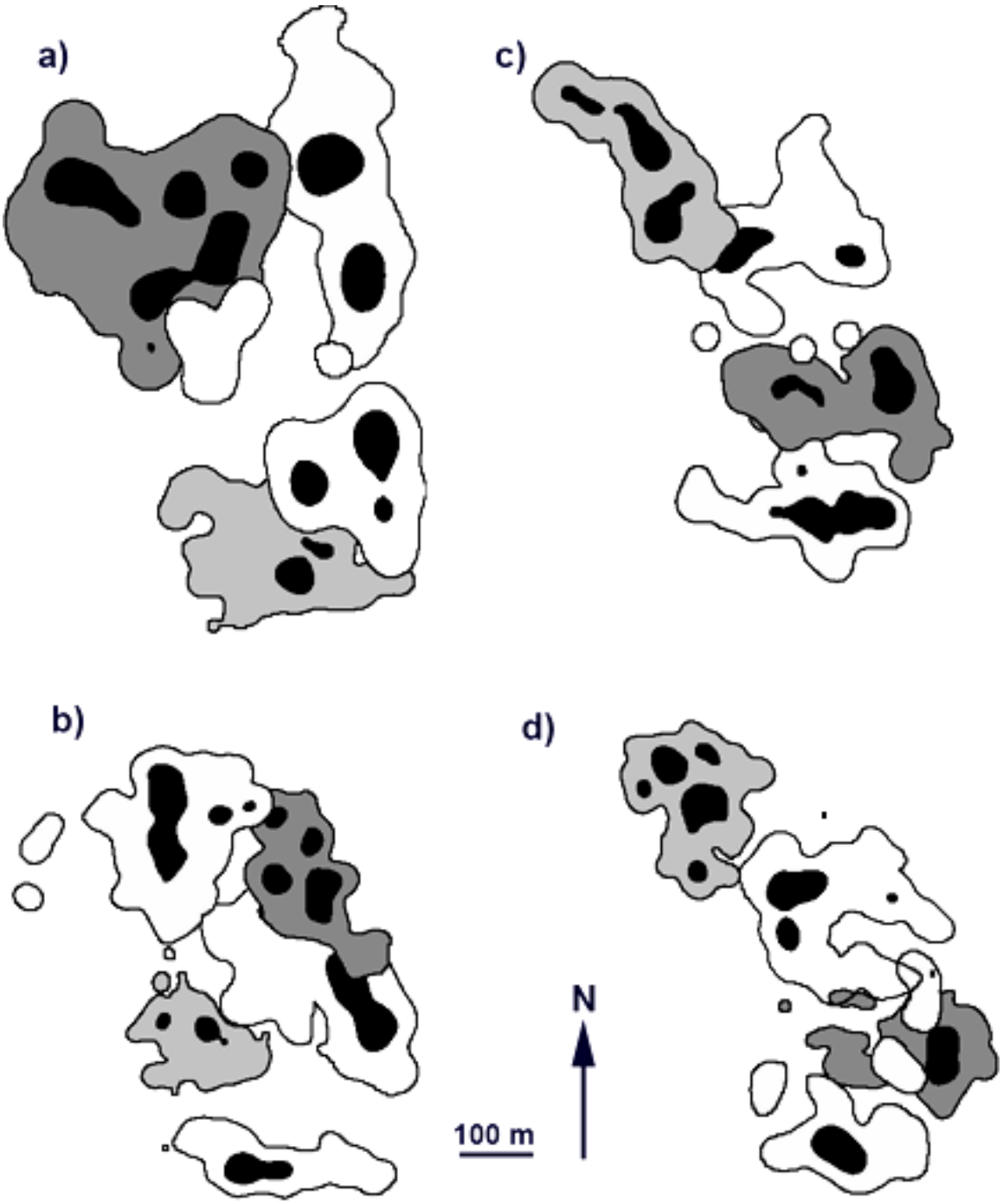
Figure 4. a) Male and b) female kernel home ranges in selectively logged forest in 2003, and c) male and d) female kernel home ranges in selectively logged forest in 2004. Black areas represent 50% kernel ranges and lighter areas represent 95% kernel ranges (pg. 50).

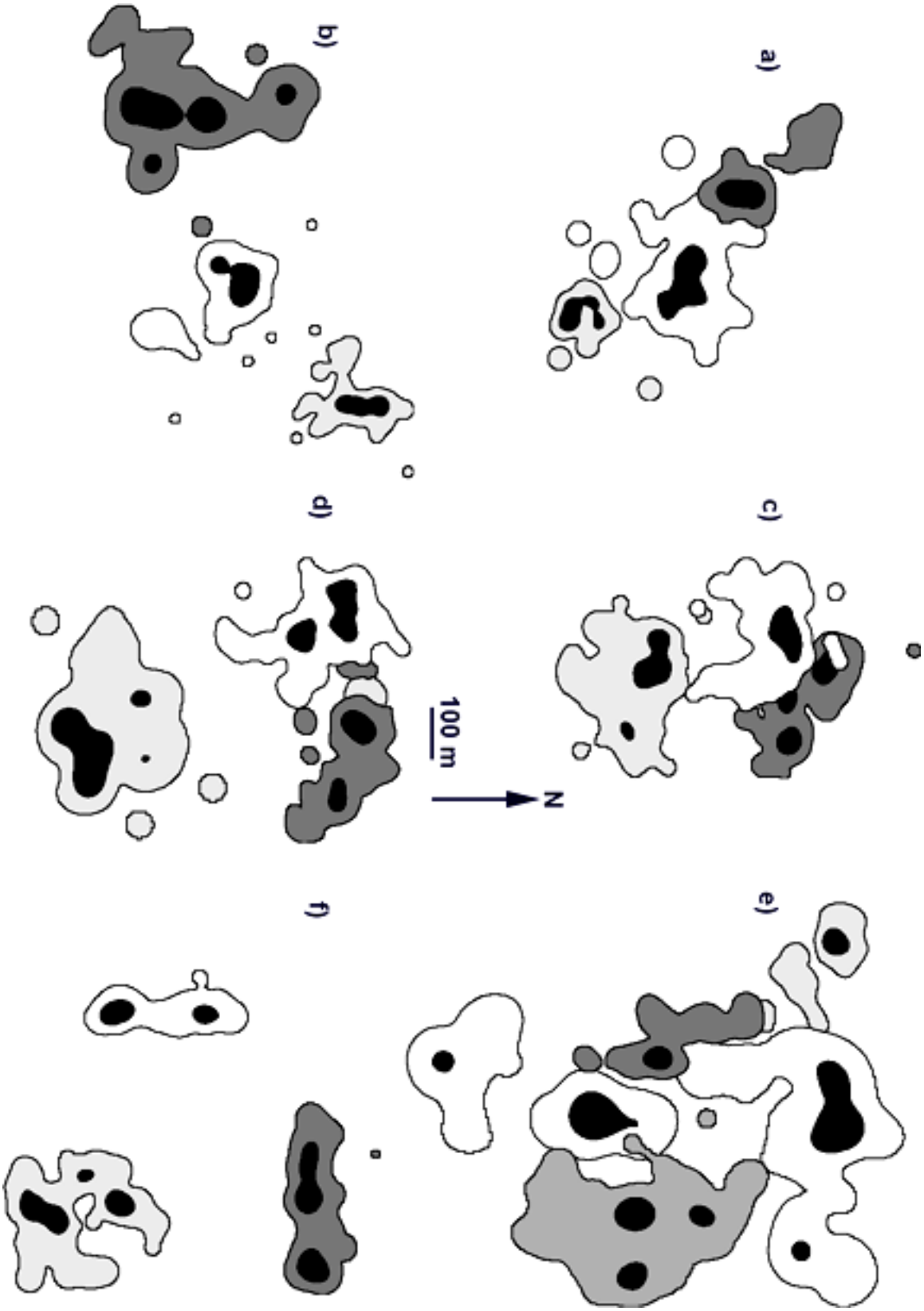
Figure 5. Mean dry weight of fruit collected per trap at the primary (white circles) and logged forest site (black circles) in 2003. Error bars represent ± one standard error of the mean (pg. 51).

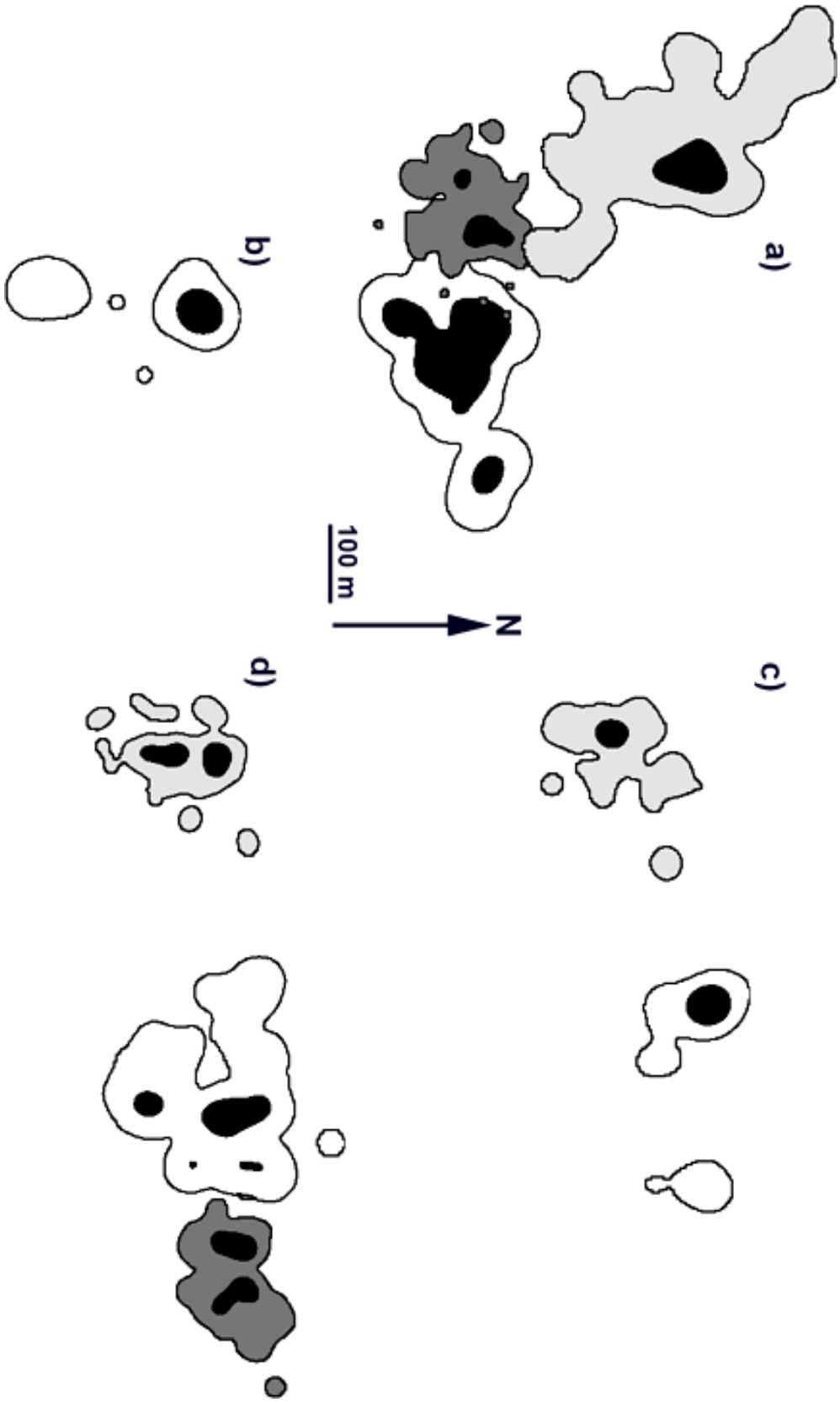
Figure 6. Relationship between mean daily distance and home range area for males (white circles, dashed line) and females (black circles, solid line; pg. 52).

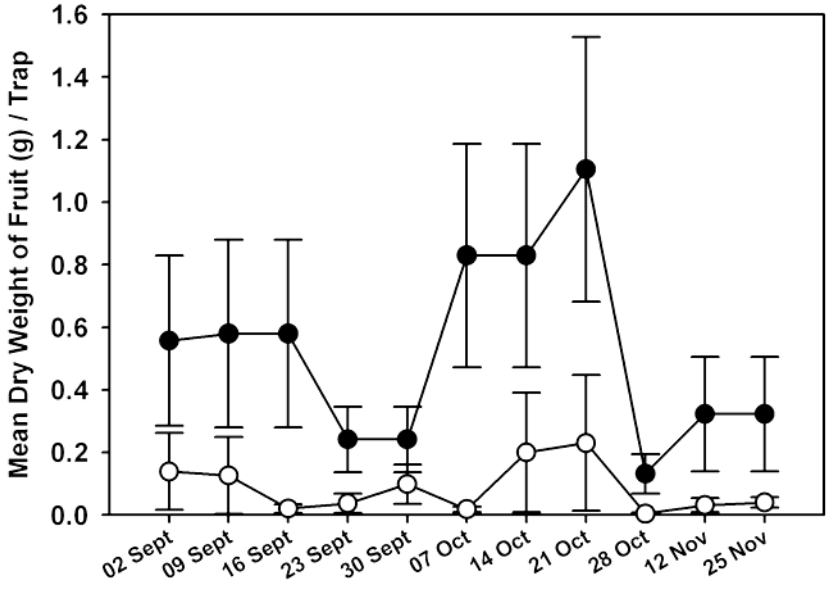
FIGURES

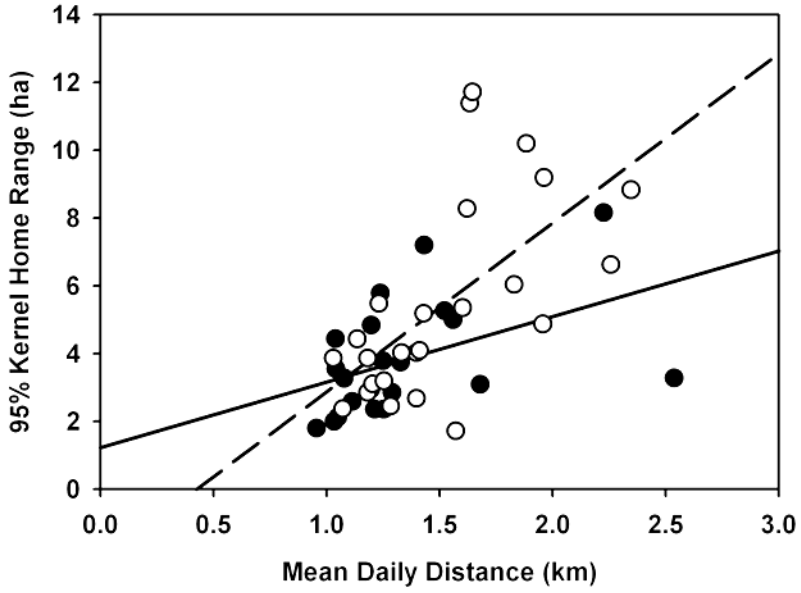

a) 1990 Masting Forest
b) 1991 Post-masting Forest
c) 2003 Primary Forest
d) 2004 Primary Forest
N
100 m
e) 2004 Logged Forest


a)
c)
b)
d)
N
100 m


(a)
(b)
(c)
(d)
(e)
(f)
100 m
N


(a)
(b)
(c)
(d)
100 m
N


Mean Dry Weight of Fruit (g) / Trap
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
02 Sept
09 Sept
16 Sept
23 Sept
30 Sept
07 Oct
14 Oct
21 Oct
28 Oct
12 Nov
25 Nov


95% Kernel Home Range (ha)
Mean Daily Distance (km)
0
2
4
6
8
10
12
14
0.0
0.5
1.0
1.5
2.0
2.5
3.0

CHAPTER II

Extra-pair paternity in a behaviorally monogamous tropical mammal, the large treeshrew (*Tupaia tana*)

ABSTRACT

Monogamy is rare in mammals (< 5% spp.), but occurs in greater frequency among primates (15%) and their close relatives, the treeshrews (100%; Order: Scandentia). Two genetic studies of parentage in monogamous primates revealed high rates of extra-pair paternity (EPP), but to date parentage has not been studied in a treeshrew species. I analyzed the genetic parentage of 24 offspring from two populations of large treeshrews (*Tupaia tana*) in Sabah, Malaysia (NE Borneo) using seven autosomal microsatellite loci and one mitochondrial DNA marker. Over 40% of young were sired by males that were not the presumed partner of the mother, and three litters exhibited evidence of multiple paternity. However, comparative analysis indicated that the high rate of EPP in *T. tana* is not associated with intense sperm competition. Relative testis size of treeshrews was similar to testis size in 22 primate species with uni-male mating systems, but smaller than 44 primates with multi-male mating systems. After factoring out the effects of body mass and phylogeny, I also found that the evolution of multi-male mating systems was significantly associated with the evolution of larger testis size. Male-female pairs of *T. tana* occupy joint territories but forage and sleep alone ("asocial monogamy"), and I argue that this form of behavioral monogamy renders mate guarding ineffective. As a

result, both males and females may seek extra-pair mating. Previously unrecognized sociobiological similarities to ancestral nocturnal prosimians indicate that treeshrews are an appropriate behavioral model for early primate evolution.

INTRODUCTION

The claim that 93% of avian species breed monogamously (Lack 1968) has been soundly refuted by evidence supporting Trivers' (1972) prediction that males and females should exhibit behavioral adaptations for extra-pair mating. An overwhelming 86% of the 130 behaviorally monogamous bird species studied by 2002 exhibited extra-pair paternity (EPP) in greater than five percent of offspring (mean = 11% of offspring and 19% of broods, Griffith et al. 2002). Hypotheses for the adaptive function of EPP abound, but predictions of female choice for genetic benefits have received the most empirical support. Females may seek EPP to obtain compatible viability genes (e.g. Johnsen et al. 2000), obtain "good genes" that increase the fitness of their offspring (e.g. Sheldon et al. 1997), or maximize the genetic diversity of their offspring (e.g. Foerster et al. 2003). Observations that EPP is less common in genetically depauperate island populations (Griffith 2000) and more common in genetically diverse populations (Petrie et al. 1998) provide additional support for genetic benefits. However, some large-scale studies have failed to detect any genetic benefit of EPP (Schmoll et al. 2003), and comparative analysis indicates that negative direct selection caused by reduced paternal care may be more important than genetic benefits in explaining variation in EPP among avian taxa (Arnqvist & Kirkpatrick 2005).

In contrast to birds, monogamy is generally rare in mammals (3-5% spp., Kleiman 1977), but occurs at greater frequency among canids, rodents, the Euarchonta (treeshrews, dermopterans, and primates), and bats (McCracken & Wilkinson 2000). The prevalence and adaptive function of EPP in behaviorally monogamous mammals is not well characterized, and recent studies have produced contrasting results. Mating exclusivity and genetic monogamy in some rodent species (California mouse, *Peromyscus californicus* and oldfield mouse, *P. polionotus*, Ribble 2003; Malagasy giant jumping rat, *Hypogeomys antimena*, Sommer & Tichy 1999) may occur because males provide care that is necessary for female reproduction or enhances offspring survival. However, at least three species with paternal care exhibit EPP (44% in the fat-tailed dwarf lemur, *Cheirogaleus medius*, Fietz et al. 2000; 10% in the African wild dog, *Lycaon pictus*, Girman et al. 1997; 25% in the island fox, *Urocyon littoralis*, Roemer et al. 2001), and most behaviorally monogamous species do not exhibit direct paternal care (Komers & Brotherton 1997). Three genetic studies on behaviorally monogamous mammals without paternal care have recorded EPP rates from 19-57% (alpine marmot, *Marmota marmota*, Goossens et al. 1998; fork-marked lemur, *Phaner furcifer*, Schülke et al. 2004; allied rock wallaby, *Petrogale assimilis*, Spencer et al. 1998), whereas exclusive mating has been confirmed using genetic data for only one such mammal (Kirk's dik-dik, *Madoqua kirkii*, Brotherton & Rhodes 1996).

Experimental studies have also confirmed that mammals with substantial behavioral and physiological adaptations for monogamy (prairie voles, *Microtus ochrogaster*, Carter et al. 1995) exhibit high rates of EPP (multiple paternity in 56% of litters, Solomon et al. 2004). These results suggest that EPP may be as prevalent in

behaviorally monogamous mammals as in birds, especially given the rarity of direct paternal care in mammals. The adaptive function of EPP has not been firmly established for any mammal species, but most previous studies have argued that females choose males of superior genetic quality (e.g. territory-holding males in fat-tailed dwarf lemurs, *C. medius*; males with longer arms in allied rock wallabies, *P. assimilis*). Protection from infanticide through paternity confusion has also recently been offered as a general explanation for multiple mating in female mammals, including some behaviorally monogamous species (Wolff & Macdonald 2004).

Variation in pair bonding may also influence mating patterns. Behaviorally monogamous mammals may form associated pairs that maintain proximity and show clear spatial association, or dispersed pairs that occupy a joint territory but are not spatially associated during periods of activity ("asocial monogamy", Chapter 1). High EPP rates have been predicted in species that exhibit dispersed pair-living because effectiveness of mate guarding may be reduced (van Schaik & Kappeler 2003). Results from the nocturnal lemurs *C. medius* and *P. furcifer* support this prediction, although nocturnality, female dominance over male partners, and highly seasonal reproduction in these species may reduce the effectiveness of mate guarding more than pair dispersion *per se* (Schülke & Ostner 2005).

This study examines the genetic mating system of the behaviorally monogamous large treeshrew, *Tupaia tana*, in Sabah, Malaysia (NE Borneo). Large treeshrews may exhibit high rates of EPP because they live in dispersed pairs, do not share sleeping sites, and do not appear to engage in substantial pair-bonding behaviors (Chapter 1, Emmons 2000). However, large treeshrews provide an interesting contrast to nocturnal prosimians

that form dispersed pairs because treeshrews are diurnal and breed relatively asynchronously. This study also provides the first genetic parentage analysis for a monogamous mammal that inhabits tropical rainforests. EPP in tropical birds is generally uncommon, possibly due to asynchronous breeding limiting the opportunity for mate assessment, or relatively larger territories and lower breeding densities than in temperate environments (Stutchbury & Morton 2001; Fleischer et al. 1997).

Genetic studies of birds and mammals have revealed that behaviorally monogamous species exhibit a diverse range of genetic mating systems. Females in pairs could copulate exclusively with their partner, mate with one or a few extra-pair males, or mate promiscuously with several males from surrounding territories. Detecting EPP and multiple paternity in large treeshrews would not necessarily indicate the extent of multiple mating and sperm competition, because their litter size is only two. To examine the potential for sperm competition in *T. tana*, I examine relative testis size and behavioral monogamy in two treeshrew and 66 primate species using both species data and phylogenetically independent contrasts. Relative testis size is a reliable predictor of sperm competition (Gage & Freckleton 2003), and sperm competition is positively associated with multi-male mating systems in multiple taxa (reviewed in Parker et al. 1997; Harcourt et al. 1995). The mating system and relative testis size of *T. tana* are discussed in the context of primate social evolution because treeshrews (Order: Scandentia) are one of two most likely sister taxa to primates (along with Dermoptera, Murphy et al. 2001), and share key sociobiological characteristics with ancestral strepsirrhine primates (Chapter 1).

METHODS

Study populations and designation of behavioral pairs

I studied a population of large treeshrews in primary lowland rainforest in the Danum Valley Conservation Area, Sabah, Malaysia (4°58´N, 117°48´E) from August to December 2002-2004, and a second population in selectively logged forest in the Malua Forest Reserve (5°5´N, 117°38´E) from September to December 2003-2004. I trapped large treeshrews at each site with locally-made wire mesh traps placed every 25 m along two 500 m transects, but in 2004 placed two additional 500 m transects at the logged site to increase captures. I conducted four-day trapping sessions every 3-4 weeks during the study period; traps were opened at 0600h and checked twice daily at 1030h and 1500h. Captured animals were transferred to cloth bags, weighed, and sedated with a ketamine hydrochloride injection. I measured hind foot length, injected animals with a subdermal passive integrated transponder (Biomark, Inc, Boise, ID) for permanent identification, and clipped a tissue sample from the upper ear. Tissue samples were preserved in 95% ethanol and stored at 4°C. If individuals were in good condition and weighed more than 180 g, then I fitted them with radio collars to identify behavioral pairs. Full details on the study site, trapping, and radiotelemetry methods can be found in Chapter 1.

Behavioral pairs of treeshrews occupy joint territories that they defend against same-sex conspecifics, but typically forage solitarily. Radio tracking of 46 individuals revealed that *T. tana* form dispersed pairs across a range of ecological conditions (Chapter 1). Having previously described the location and shape of adult territories for each site in each year using radiotelemetry data and spatial analyses, I designated behavioral pairs of *T. tana* when at least 50% of a female's territory was contained within

the territory of a single male (Chapter 1). No individuals had more than one behavioral partner, although most individuals slightly overlapped extra-pair territories. Only two individuals (F14 and M35) persisted for more than one study period, but the spatial arrangement of home ranges was similar across years, even when occupied by different individuals. Incomplete sampling or radio collar failure prevented designation of pairs for all adults, particularly in primary forest in 2002 and selectively logged forest in 2003. When radio-tracking data were not available, I used trapping locations to identify presumed mates ($N = 3$ behavioral fathers: M03, M07, and M08, Table 1). The presumed mates identified using this latter method were trapped on a known female's home range multiple times, and were surrounded by same-sex home ranges identified through radiotelemetry (i.e. process of elimination aided designation of these males).

The length of the female receptive period has not been described for wild *T. tana*, but has been reported as only one to three hours for captive *T. belangeri* (Martin 1968). *T. tana* females can produce up to nine litters annually in captivity, but in the wild have one to three litters per year during and shortly after peak annual resource abundance (Emmons 2000). Treeshrews also exhibit a unique maternal care system whereby they deposit their young in a nest that they subsequently visit only once every 48 hours for intense nursing bouts (Martin 1966). As a result, I could identify juveniles only after weaning when they were trapped outside the nest. Juveniles were identified by their small size (mass < 180 g based on growth curve in Emmons 2000) and the presence of milk teeth or newly-erupted unworn adult teeth. I trapped 15 juveniles in primary forest and eight juveniles in selectively logged forest during the study period (Table 2).

Genetic parentage analysis and relatedness calculations

DNA was extracted from ear tissue samples using Qiagen DNeasy tissue kits (Qiagen, Valencia, California, USA). Seven previously-described microsatellite DNA loci named JS22, JS132, JS183, JS188, JS196, SKTg19, and SKTg22 were amplified using the PCR conditions in Appendix 1. Fluorescently-labeled alleles were separated on an Applied Biosystems 3100 DNA Analyzer and sized and scored using Genotyper 2.5 (Applied Biosystems, Foster City, California, USA). Locus JS183 exhibited a homozygote deficiency consistent with the presence of null alleles (Appendix 1), so I ran all analyses with and without this locus because null alleles can substantially influence molecular parentage analyses (Dakin & Avise 2004).

I also PCR-amplified a 602 bp segment of the mitochondrial DNA (mtDNA) control region to limit the number of candidate mothers based on shared mtDNA sequences. Primers were designed from conserved segments of the control region in the northern treeshrew, *Tupaia belangeri*, and the sister taxon to treeshrews, the Malayan colugo, *Cynocephalus variegatus* (GenBank Accession Nos. AF217811 and AJ428849, respectively, Murphy et al. 2001; Schmitz et al. 2000; Arnason et al. 2002), using the Primer3 computer program (Rozen & Skaletsky 2000). PCR amplification was performed in 9 µl volumes containing 1 µl template DNA, 0.125 U *Taq* polymerase (Invitrogen), 1X PCR buffer (Invitrogen), 0.3 mM of each dNTP, 2.5 mM $MgCl_2$, and 0.55 µM of each primer (forward primer JMSTbel386 5′-ACCTCCGTGAAATCAGCAAC-3′ and reverse primer JMSTbel1110 5′-TTCTTGTTTTTGGGGTTTGG-3′). PCR was performed on a Peltier thermocycler programmed for 30 amplification cycles with denaturation at 95° C for 1 min, annealing

at 55° C for 1 min, and extension at 72° C for 1 min. I sequenced the forward strand of the PCR product using the BigDye Terminator 3.1 and a 3100 DNA Analyzer (Applied Biosystems). Sequences were edited and aligned using Sequencher 4.1.2 (Gene Codes, Ann Arbor, Michigan, USA) and Bioedit 7.0.4.1 (Hall 1999).

Parentage likelihood analyses were conducted separately for treeshrews from the primary and selectively logged forest sites using Cervus 2.0 (Marshall et al. 1998; Slate et al. 2000). All Cervus analyses were based on a simulation with 10,000 cycles assuming 5 candidate parents, complete parental sampling and genotyping, and a 1% genotyping error rate. This simulation predicted a parentage assignment success rate of 74% at the strict criterion and 99% at the relaxed criterion when neither parent is known, and 100% for both criteria when one parent is known. Neither parent was known *a priori* for any offspring, so I conducted a stepwise parentage analysis. First, I assigned genetic mothers to offspring when the certainty calculated by Cervus for one female exceeded 80% (relaxed criterion) or 95% (strict criterion). I limited the number of candidate mothers for each offspring in the maternity analysis based on shared mtDNA control region haplotypes, because Cervus is more successful at assigning parentage when there are fewer candidate parents. Thirteen mtDNA haplotypes defined by ten segregating sites were identified from a relatively conserved 324 bp segment of the 602 bp control region sequence (Table 1). Genetic mothers were not assigned to all offspring after an initial analysis where candidate mothers were limited by shared haplotypes. Therefore, I re-ran the analysis with all adult females as candidate mothers for offspring not successfully assigned genetic mothers during the first analysis. The results from this mitochondrial screening procedure did not identify different mothers than a separate Cervus analysis

that included all maternal candidates, but did provide higher likelihood values for some assigned mothers.

Mothers assigned to offspring were then carried over to the paternity analysis as known parents, and genetic fathers were assigned at either the strict or relaxed criterion. Offspring assigned both parents were designated as the result of either intra-pair (IPP) or extra-pair (EPP) paternity based on whether their genetic father was also their behavioral father as defined above. I also recorded the number of loci excluding the behavioral father as the genetic father for each offspring. In cases where multiple loci excluded the behavioral father but a genetic sire was not assigned in the likelihood parentage analysis, I designated parentage as EPP. When no loci excluded the behavioral father but a genetic sire was not assigned, I designated parentage as IPP. When I omitted locus JS183 from the analysis due to possible null alleles, I found reduced support for some parentage assignments but no support for alternative parental relationships. The only exception was the assignment of two potential sires at 80% certainty for offspring f28, but neither could be definitively assigned. Once offspring were assigned to genetic parents, I tested for a difference in EPP rates between primary and logged forests using a Pearson's χ^2 test. I used a *t* test and *F* test of unequal variances, respectively, to examine whether mean and variance in the number of offspring sired by males was significantly different from the mean and variance in offspring assigned to females.

Female *T. tana* give birth to litters of two offspring, so when littermates were trapped I examined the possibility of multiple paternity using the parentage analyses above and genetic estimates of pair-wise relatedness. I used the program ML-RELATE (Kalinowski et al. in press) to calculate maximum likelihood estimates of pair-wise

relatedness between genetic mothers and offspring, genetic fathers and offspring, putative full siblings, and putative half-siblings identified by the parentage analyses. Maximum likelihood estimates of relatedness are generally more accurate than other estimators at determining specific relationships (Milligan 2003), and this particular implementation accounts for the influence of null alleles on relatedness calculations (7% null alleles estimated for locus JS183, Kalinowski & Taper unpublished manuscript). Lower pair-wise relatedness values for littermates than for parent-offspring or full-sibling dyads was considered evidence in favor of multiple paternity.

Testis size analysis

To examine the potential for sperm selection in treeshrews and primates, I collated primate species data on testis size and body size from earlier reviews of all mammals ($N = 14$ spp., Gage & Freckleton 2003), all primates ($N = 28$, Harcourt et al. 1995), and strepsirrhine primates ($N = 24$, Schülke et al. 2004). Testis size for male *T. tana* ($N = 15$ individuals) and the plain treeshrew (*Tupaia longipes*, $N = 3$) trapped during this study were calculated using the formula $1/6 \times \pi \times \text{Length} \times \text{Width}^2$ (Hosken 1998). Only species values for which the behavioral mating system could be identified were used. The mating system for each species was designated as either behaviorally monogamous, polygynous, or multi-male. Mating system designation was based on the information in the testis size references above or Komers and Brotherton (1997). Following Schülke et al. (2004), species where males are solitary and dispersed were classified as exhibiting multi-male mating systems. I used analysis of covariance

(ANCOVA) with log body size as the covariate to examine whether log testis size differs between mating systems.

Species data cannot be treated as statistically independent, because species are related through descent from common ancestors (Felsenstein 1985). Hence, I also used CAIC (Comparative Analysis by Independent Contrasts) v. 2.6.9 (Purvis & Rambaut 1995), to convert species data into phylogenetically independent contrasts. I used a recent, highly resolved supertree phylogeny of all primates with branch lengths (Vos & Moores in press). To remove the effects of body mass on testis size, I first calculated the independent contrasts of log body mass and log testis size using the CRUNCH algorithm. I then calculated the least squares regression equation forced through the origin, and used this regression formula to calculate residuals from the raw testis size data. These residual values were then tested against mating system categories using the BRUNCH algorithm in CAIC. The BRUNCH algorithm requires a dichotomous categorical variable, so monogamy and polygyny were lumped together as uni-male mating systems and compared to multi-male mating systems. I used a *t* test to examine whether these categorical contrasts were significantly above zero, as predicted if the evolution of multi-male mating systems is associated with the evolution of larger testis size in primates (see CAIC manual, Purvis & Rambaut 1995). JMP version 5.0.1.2 (SAS Institute 2003) was used for all statistical analyses.

RESULTS

Both genetic parents could be assigned for 10 out of 15 (67%) offspring in primary forest with at least 80% confidence, although behavioral fathers were known in only 8 of these

cases (Table 2). Five out of 15 genetic mothers were assigned at the strict (95%) confidence level, whereas 9 out of 10 genetic sires were assigned at the strict confidence level once genetic mothers were assigned. In selectively logged forest, both genetic parents were assigned for four out of nine (44%) offspring (Table 2). Behavioral fathers were identified for five of these cases, but in one case the genetic mother was not assigned (offspring m48). Five out of six genetic mothers and four out of five genetic fathers were assigned at the strict confidence level.

The parentage analyses identified EPP among treeshrews in both primary and selectively logged forest. Of the 8 offspring in primary forest for which both genetic parents and the behavioral father were assigned, four resulted from EPP and four from intra-pair paternity (50% EPP, Table 2). EPP was suspected in five additional cases where either a behavioral father was not identified, or paternity by the behavioral father was excluded by multiple loci (64% EPP overall in primary forest if these five are included). Of the four offspring in selectively logged forest with complete parentage information, two resulted from EPP and two from IPP (50% EPP, Table 2). Three additional cases of IPP were suspected: two offspring mothered by F38 but for which behavioral and genetic fathers were not identified, and one offspring sired by M35 for which a genetic mother could not be identified (f40, m46, and m48; 29% EPP overall in logged forest if these three are included).

The behavioral father was not excluded by any loci in eight cases of IPP, whereas the behavioral father was excluded by two loci in seven cases of EPP (Table 2). EPP rates were not significantly different between sites if the incomplete parentage assignments were included (Pearson's $\chi^2_{1,19} = 2.39$, $P = 0.12$) or omitted (Pearson's $\chi^2_{1,10}$

= 0.001, $P = 0.99$). The overall rate of EPP for both sites was 46%, or 52% if the incomplete parentage assignments were included. Female adults were assigned significantly more genetic offspring ($N = 12$, mean ± SE = 1.75 ± 0.28 offspring; $t_{28} = 2.45$, $P = 0.02$) than male adults ($N = 18$, mean ± SE = 0.83 ± 0.23), but variance in reproductive success was not significantly different between the sexes ($F_{1,28} = 0.24$, $P = 0.63$).

Average pair-wise relatedness (mean ± SE) between genetic mothers and offspring ($N = 18$, $r = 0.36 \pm 0.04$), genetic fathers and offspring ($N = 13$, $r = 0.36 \pm 0.05$), and full-siblings ($N = 6$, $r = 0.37 \pm 0.05$) was more than twice the average recorded for half-siblings ($N = 12$, $r = 0.12 \pm 0.04$). Three putative littermate pairs were identified when two offspring shared the same genetic mother, were trapped within a few days of each other and were similar in mass at time of capture. In two cases (m11-m14 in primary forest, $r = 0.09$; f33-m43 in selectively logged forest, $r = 0.0$) littermates had different genetic sires and low pair-wise relatedness values, suggesting that multiple paternity occurs in *T. tana*. The offspring pair f22-f28 ($r = 0.0$) in primary forest may represent another case of multiple paternity, although the genetic sire of f28 was not assigned. These results indicate a minimum multiple paternity rate of 32%, assuming no other cases of multiple paternity in incompletely sampled litters.

In most cases of EPP, extra-pair sires occupied territories that were directly adjacent to their extra-pair mate in that year (Figure 1). The three exceptions all occurred in primary forest in 2002, and included male M06 that fathered offspring f02 with female F06 in 2002 at the primary forest site. The boundary of M06's territory was separated from F06's territory by 280 m at their closest point, and another pair's territory (F08—

M08) was located between them. The other two exceptions were extra-pair offspring sired by M01 and M10, males that sequentially occupied a territory where no adult female was captured in 2002 (Figure 1). Behavioral partners were not captured for four out of seven extra-pair males (Figure 1; M01, M06, M10, M50), and one female that mated with males on other territories (Figure 1; F06).

Relative testis size of behaviorally monogamous species, including *T. tana* and *T. longipes*, was consistently smaller than relative testis size in species with multi-male mating systems (Figure 2). ANCOVA confirmed that log testis size increased with log body size in all species, ($F_{1,67} = 39.4$, $P < 0.0001$), and there was a significant differences in testis size ($F_{1,67} = 15.9$, $P < 0.0001$) between species with behaviorally monogamous (adjusted mean ± SE = 3.29 ± 0.09), polygynous (3.50 ± 0.33), and multi-male mating systems (3.86 ± 0.05). The interaction between body mass and mating system was not significant ($F_{1,67} = 0.04$, $P = 0.96$), indicating that the regression lines for monogamous, polygynous, and multi-male mating systems did not have significantly different slopes (Figure 2).

The linear regression forced through the origin of the independent contrasts of log testis size on the contrasts of log body mass was statistically significant (Figure 3; $N = 51$ contrasts; log testis size = 0.51 X log body mass, $F_{1,50} = 11.54$, $P = 0.001$). Analysis of phylogenetically independent contrasts of residual testis size (controlled for body mass) and behavioral mating system indicated that the evolution of multi-male mating systems is significantly associated with the evolution of larger testis size (Figure 4; $N = 14$ contrasts, mean ± SE = 0.11 ± 0.02; $t_{1,13} = 6.96$, $P < 0.0001$).

DISCUSSION

Genetic mating system of the large treeshrew

Genetic analysis of parentage in the large treeshrew revealed one of the highest rates of EPP recorded for a behaviorally monogamous mammal. Only EPP rates reported for the lemurs *C. medius* (44%, Fietz et al. 2000) and *P. furcifer* (four out of seven offspring, Schülke et al. 2004) are of comparable magnitude. We also found evidence for multiple paternity in large treeshrews, indicating that female *T. tana* may mate with more than one male in a single breeding period.

Extra-pair males fertilized females that resided on neighboring territories in most cases, but in a few instances extra-pair mating occurred between individuals separated by another pair's territory. A majority of extra-pair males did not have known behavioral mates (four out of seven, Figure 1), suggesting that male *T. tana* instigate extra-pair mating, particularly when they may not have the option of mating within a behaviorally monogamous pair. Emmons' (2000) multiple observations at the same site of short-term male forays to visit extra-pair females also suggest male initiation of extra-pair mating.

Testis size, pair-living, and EPP

Comparative analysis of testis size revealed that primates with multi-male mating systems have relatively larger testes than behaviorally monogamous or polygynous treeshrews and primates (Figures 2-4). These results were independent of body mass and phylogeny, and generally agree with previous analyses (all primates, Harcourt et al. 1995; strepsirrhine primates, Schülke et al. 2004; Kappeler 1997). However, previous analyses either did not include treeshrews, did not account for phylogenetic dependence, or used a

less resolved primate phylogeny (especially for prosimian clades of interest in this study, Purvis & Webster 1999; Purvis 1995). Large relative testis size is a reliable predictor of sperm competition in species with multi-male mating (Gage & Freckleton 2003; Parker et al. 1997). However, high EPP rates in large treeshrews and two nocturnal lemurs with small testes indicate that greater sperm competition does not necessarily result from extra-pair copulations. We could not directly observe copulations in *T. tana*, but small relative testis size in this species suggests that females do not copulate promiscuously during one receptive period.

Discordance between high EPP rates and small testis size could result from evolutionary constraints on testis size or abnormally high population densities due to environmental degradation (Schülke & Ostner 2005). Sperm morphometry, and particularly sperm size, could also be more important than sperm number for fertilization in treeshrews and other mammals. Sperm length is positively correlated with testis size in mammals, although the relationship is phylogenetically dependent (Gage & Freckleton 2003). I argue below that high population density is not responsible for EPP in *T. tana*, but additional data on testis size and sperm morphometry in treeshrews are needed before other explanations can be ruled out. Fewer mates and lifetime breeding opportunities compared with polygynous or promiscuous primates may be more likely explanations for small testis size in *T. tana*. Only two individuals existed on the study site for more than one year (Figure 1), and wild treeshrews may have only one to three reproductive opportunities in their lifetime (Emmons 2000).

Explanations for EPP in large treeshrews

High rates of EPP in behaviorally monogamous species may result from specific ecological conditions, such as high breeding density or synchrony, or adaptive evolutionary benefits to females (Griffith et al. 2002). Adaptive explanations for EPP can be further divided into direct benefits provided by extra-pair males, and indirect benefits from genetic quality or genetic variation. Direct benefits from paternal care, improved foraging, infanticide prevention, or predation prevention are largely precluded by the reproductive biology and ranging patterns of *T. tana* (Chapter 1). Extensive radio tracking showed that female *T. tana* do not spend significant time foraging or engaged in other activities on extra-pair home ranges, so they cannot receive direct benefits from extra-pair males (Chapter 1). However, given the number of unpaired males that sired extra-pair young (Figure 1), the possibility that behaviorally monogamous females mate with extra-pair males to avoid continuous male harassment (Wolff & Macdonald 2004) cannot be ruled out for large treeshrews.

The prevalence of EPP in *T. tana* raises the question of the prevalence and effectiveness of male mate guarding. Brotherton and Komers (2003) argued that behavioral monogamy in mammals can primarily be explained by the benefits of male mate guarding strategies, and predicted that most female mammals do not seek extra-pair copulations because of the costs of aggressive conflicts (e.g. Kirk's dik-dik, *M. kirkii*, Brotherton & Manser 1997). However, *T. tana* and many nocturnal prosimians form dispersed pairs, presumably to avoid foraging competition (Chapter 1, Schülke & Ostner 2005). Avoidance behaviors may render mate guarding ineffective in these species, leading to high rates of EPP. Mate guarding may also be unsuccessful when extra-pair

males have good information on the estrous state of neighboring females. Female *T. tana* forage solitarily and have relatively long day ranges for their home range size (Chapter 1). As a result, males may not be very successful at over-marking the scent marks of their female partners. Male *T. tana* will likely maximize their reproductive success if they mate with their behavioral partner but also pursue extra-pair copulations, rather than making large temporal and energetic investments in mate guarding.

The high rates of EPP recorded for *T. tana* populations in tropical rainforests are at odds with the observation that EPP is uncommon in tropical birds (Stutchbury & Morton 2001). Relatively asynchronous breeding in tropical birds may limit the abilities of males to pursue EPP and females to assess extra-pair males. Low EPP rates may further explain the relatively smaller testis size in tropical vs. temperate songbirds (Stutchbury & Morton 1995). However, the correlation between EPP and breeding synchrony in birds is difficult to separate from other causal factors (Griffith et al. 2002), and smaller relative testis size does not necessarily imply that EPP does not occur (this chapter and Schülke et al. 2004). Only three previous studies have been conducted on monogamous mammals in the tropics, and all three were conducted on sympatric species in a dry deciduous forest in Madagascar: two lemurs that exhibited high rates of EPP and very short breeding seasons (two weeks, Schülke et al. 2004; Fietz et al. 2000), and a genetically monogamous rodent with substantial male parental care (Sommer & Tichy 1999). I studied treeshrews from August to December to maximize offspring captures, because Emmons (2000) recorded the highest reproductive output for *T. tana* during these months. However, young were recorded in nearly all months of the year in Emmons' study, suggesting that *T. tana* reproduce relatively asynchronously. Breeding

synchrony thus does not adequately explain EPP in the large treeshrew, although it is possible that EPP is less common during the time period not covered in this study (January to July).

High density of breeding adults is another ecological explanation for high rates of EPP, but has not received robust support in comparative avian studies (Griffith et al. 2002). EPP was detected in an insular fox species with one of the highest population densities ever recorded for a canid, presumably because territorial proximity and limited opportunities for dispersal in an insular habitat facilitated promiscuous mating (although this population may have been abnormal due to extreme predation pressure; Roemer et al. 2001). The *T. tana* population in selectively logged forest exhibited just as much EPP as the population in primary forest, despite longer distances between neighboring pairs in logged forest than in primary forest (Chapter 1). Two sympatric tupaiids occur at lower population densities than large treeshrews (*T. longipes* and *T. gracilis*, Emmons 2000), and would provide an interesting test of the hypothesized association between breeding density and EPP.

Implications for primate social evolution

Treeshrews and the two extant dermopterans are the closest living relatives of primates (Murphy et al. 2001), and thus serve as valuable outgroups for making inferences about the ancestral primate social organization. Martin (1990) argued that treeshrews bear almost no resemblance to the extant, small-bodied (< 500 g) prosimians that are closest to the base of the primate evolutionary tree. His reasoning was based on a perceived lack of grasping feet and hind limb-dominated locomotion in treeshrews, as well as the claim that

most ancestral prosimians exhibit a dispersed harem polygyny social organization. However, recently described morphological similarities with ancestral primates (e.g. grasping hands and feet in the pentail, *Ptilocercus lowii*, and the pygmy treeshrew, *T. minor*, Sargis 2004) and behavioral similarities to cheirogaleid lemurs (Chapter 1, Schülke & Ostner 2005), indicate that treeshrews may be one of the best living models of early primates. Recent reviews including new data from cheirogaleids also indicate that dispersed monogamy and dispersed multi-male social systems are more common than dispersed harem polygyny among ancestral primates (Müller & Thalmann 2000).

The Cheirogaleidae were one of the first families to diverge from the lemur tree (Yoder & Yang 2004; 31-50 MYA, Roos et al. 2004), and thus are likely to represent the ancestral condition of lemurs and other primates (Schülke & Ostner 2005; Martin 1990). Dispersed pair-living and high rates of EPP in species from the two most basal cheirogaleid genera, *Phaner* and *Cheirogaleus* (Pastorini et al. 2001), closely resemble results from the large treeshrew (this chapter and Chapter 1). Other cheirogaleids, the basal aye-aye (*Daubentonia madagascarensis*), and lorisiformes are all nocturnal solitary foragers that live in either dispersed pairs or dispersed multi-male systems (Müller & Thalmann 2000). Given that the treeshrew lineage is basal to the nocturnal prosimian lineage, these patterns imply that dispersed multi-male mating systems evolved from a dispersed pair system. The basal pentail treeshrew, *P. lowii*, exhibits associated rather than dispersed pair-living (Emmons 2000), and some other *Tupaia* species exhibit affiliative pair-bonding behaviors in captivity (e.g. *T. belangeri*, Martin 1968) or in the wild (*T. minor* and *T. montana*, Emmons 2000). However, the mating systems of many "intermediate" treeshrew lineages between *P .lowii* and the relatively derived *T. tana*

have not been adequately studied. At the very least, associated or dispersed pair-living should be considered as equally likely as dispersed multi-male systems, and more likely than dispersed harem polygyny social systems, to characterize ancestral primate social organization.

Conclusions

This study provides the first genetic analysis of a treeshrew mating system, and the first results from a behaviorally monogamous mammal in a tropical rainforest. I detected one of the highest rates of EPP recorded for a pair-living mammal. Males may instigate most extra-pair mating, and seem to seek EPP more often when they do not have the option of mating with a female partner. The dispersed pair system of *T. tana* may render male mate guarding ineffective and lead to the high rates of EPP observed in this species. High EPP rates in treeshrews and pair-living primates were not associated with large relative testis size, implying that sperm competition is not an important evolutionary force in behaviorally monogamous treeshrews or primates. The sociobiological similarities between *T. tana* and ancestral prosimians has heretofore been unappreciated, but may have important implications for understanding social evolution in primates.

TABLES

Table 1. Frequency and characteristics of 13 mitochondrial control region haplotypes among 41 large treeshrews from primary and selectively logged forest. The haplotypes are characterized by nucleotide substitutions at 10 variable sites in a 324 bp sequence (alignment gaps and missing data excluded; pg. 75). The consensus sequence follows:

SKTCAGGGCCATTGAYTGAAGATCGCCCACACNYBKTGWCCHYKTAAATAA
GACATCTCGATGGATTCRTGACTAATCAGCCCATGCCTAACATAACTGTGSTG
TCATGCCYTTGGTATTTTTAAAATTTAGGGGTGGTATCACTCAACAGGGCCGG
GAGGCCTCGTCCCAGGCAAACTGATTGTAGCTGGACTTAACTTGAATATTCTT
TAATCGCATATAAACCATAGGGTGTAATCTTTCCATGCTCGATGGACATAACA
AATCATCAATACAGACCCAAACAYAAACCCAACCCRACGCACGTACACGTAC
ACGTACACG

Haplotype	*N*	Prop.	nt1	2	9	16	41	158	233	287	299	302
Ttdlp1	1	0.02	G	G	C	C	C	G	T	T	A	G
Ttdlp2	12	0.29	C	T	.	.	.	.	.	.	.	.
Ttdlp3	10	0.24	C	T	.	T	.	.	.	.	.	.
Ttdlp4	5	0.12	C	T	.	T	.	.	.	.	.G	.
Ttdlp5	3	0.07	C	T	.	T	.	.	.	C	.	.
Ttdlp6	1	0.02	C	T	.	T	.	A	G	.		.
Ttdlp7	2	0.05	.	T	.	T	.	.	.		.	.
Ttdlp8	1	0.02	C	.	.	T	.	.	.	.	.	.
Ttdlp9	2	0.05	.	T	.	.	.	.	.	.	.	.
Ttdlp10	1	0.02	C	T	G	T	.	.	.	.	.	.
Ttdlp11	1	0.02	C	T	.	T	A	.	.	.	.	.
Ttdlp12	1	0.02	C	T	.	T	.	.	.	.	.	A
Ttdlp13	1	0.02	.	.	.	T	.	.	G	.	G	.

Table 2. Behavioral and genetic parentage of 15 offspring in primary forest and 7 offspring in selectively logged forest from 2002-2004. Offspring f20 and m33 in logged forest are not included in the table because no parentage information could be established for them. Paternity was designated as either intra-pair (IPP), extra-pair (EPP), or unassigned, based on the number of loci excluding paternity of the behavioral father, and a maximum likelihood analysis of paternity. The likelihood analysis was based on a simulation with a genotyping error rate of 1%, and thus identified IPP for offspring m04 despite one locus excluding the behavioral father. * parentage assigned at 80% likelihood ** parentage assigned at 95% likelihood [a] behavioral father designation based solely on trapping data ? suspected parentage – one parent unassigned (pg. 77).

(table follows on next page)

Offspring	Year	Haplotype	Genetic mother	Behavioral father	No. Loci Excluding IPP	Genetic father	Paternity
Primary Forest							
f02	2002	na	F06*	undetermined	na	M06**	EPP?
f07	2002	Ttdlp2	F08**	M08	2	M01**	EPP
f09	2002	Ttdlp2	F06*	undetermined	na	M10**	EPP?
f22	2004	Ttdlp2	F26*	M40	2	M50**	EPP
f23	2004	Ttdlp4	F21*	M39	2	unassigned	EPP?
f28	2004	Ttdlp5	F26*	M40	0	unassigned	IPP?
f36	2004	Ttdlp5	F10**	M29	1	M19*	EPP
m04	2002	na	F01*	M03[a]	1	M03**	IPP
m11	2002	Ttdlp2	F04*	M07[a]	0	M07**	IPP
m14	2002	Ttdlp2	F04**	M07[a]	2	M06**	EPP
m17	2003	Ttdlp2	F14*	M19	2	unassigned	EPP?
m24	2003	Ttdlp3	F10**	M08[a]	2	unassigned	EPP?
m25	2003	Ttdlp2	F14*	M19	0	unassigned	IPP?
m37	2004	Ttdlp9	F26*	M40	0	M40**	IPP
m49	2004	na	F26**	M40	0	M40**	IPP
Logged Forest							
f33	2004	Ttdlp4	F29**	M35	0	M35**	IPP
f39	2004	Ttdlp3	F35**	M31	0	M31**	IPP
f40	2004	na	F38**	undetermined	na	unassigned	IPP?
m43	2004	Ttdlp11	F29*	M35	2	M31**	EPP
m45	2004	Ttdlp12	F29**	M35	1	M31**	EPP
m46	2004	Ttdlp3	F38**	undetermined	na	unassigned	IPP?
m48	2004	Ttdlp13	unassigned	M35	0	M35*	IPP

FIGURE LEGENDS

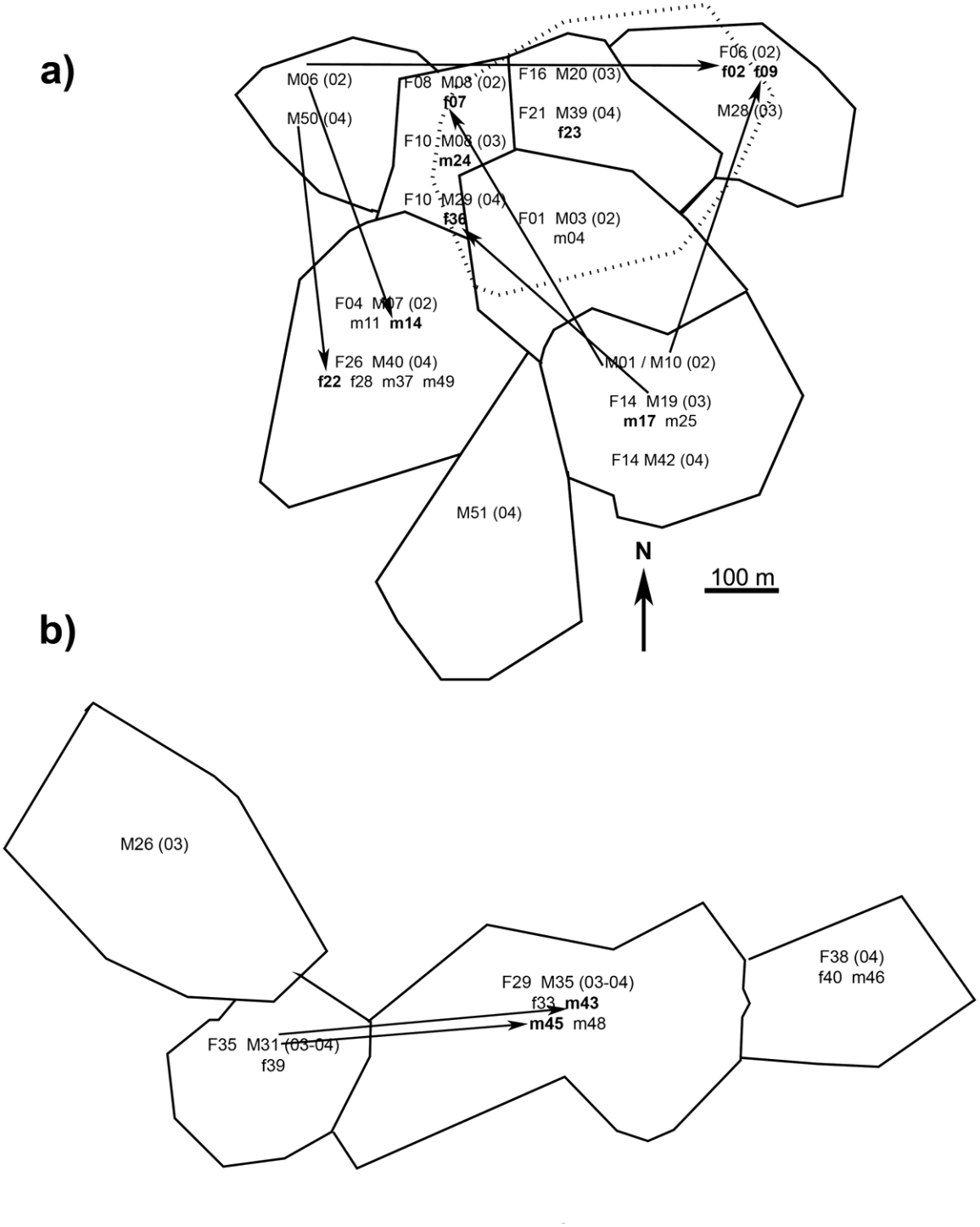
Figure 1. Schematic location of pair territories and capture sites in a) primary forest in 2002-2004, and b) selectively logged forest in 2003-2004. Note that diagram represents relative territorial arrangement; see Figures 1-4 in Chapter 1 for actual spatial overlap between territories. Identity of adult pair members is denoted by F (female) and M (male), followed by their year of residence in that territory in parentheses. Each pair's offspring are listed directly underneath their parents and denoted by f (female) or m (male). Bold offspring names denote extra-pair paternity, and arrows point from extra-pair fathers to their genetic offspring. The dashed polygon represents the anomalous schematic territory of M39 in 2004, which was much larger than other territories recorded in this study (pg. 80).

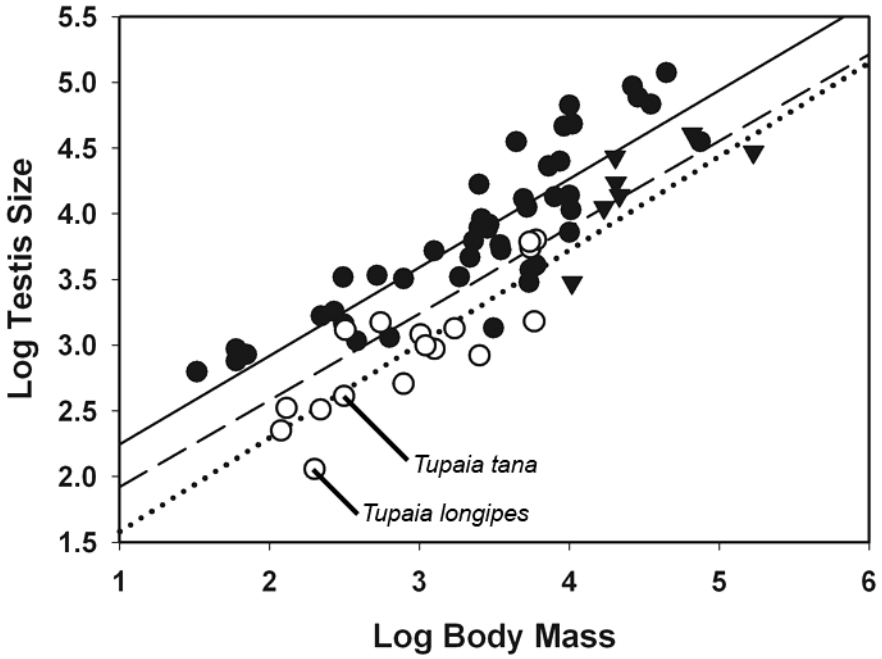
Figure 2. Relationship between log testis size and log body size in 2 treeshrew and 66 primate species. White circles with the dotted regression line correspond to behaviorally monogamous species, black triangles with the dashed regression line denote polygynous species, and the black circles with the solid regression line represent species with multi-male mating systems (pg. 81).

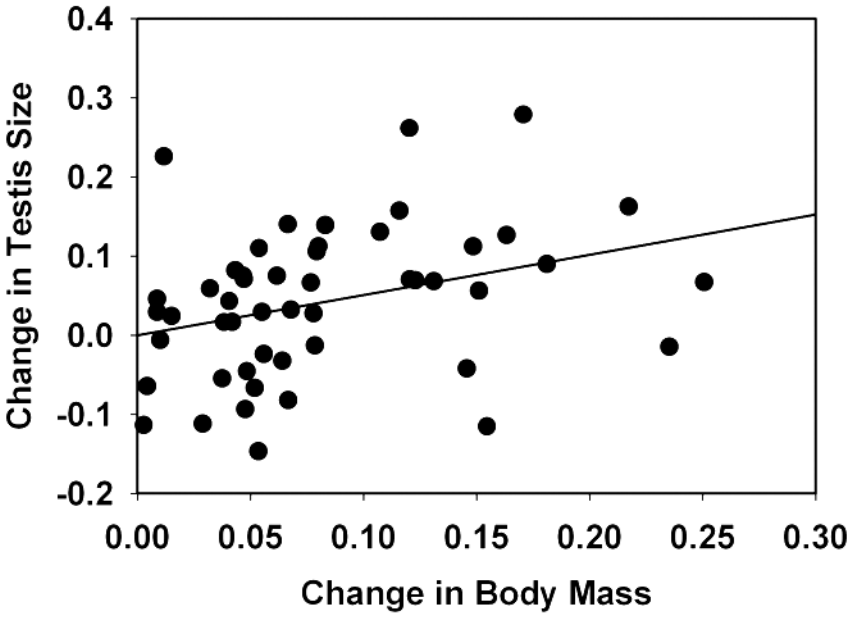
Figure 3. Independent contrasts ($N = 51$) of the evolutionary change in testis size vs. the evolutionary change in body mass in primates. Contrasts were generated using the CRUNCH algorithm in CAIC. The solid line represents the simple linear regression of change in testis size on change in body mass forced through the origin (pg. 82).

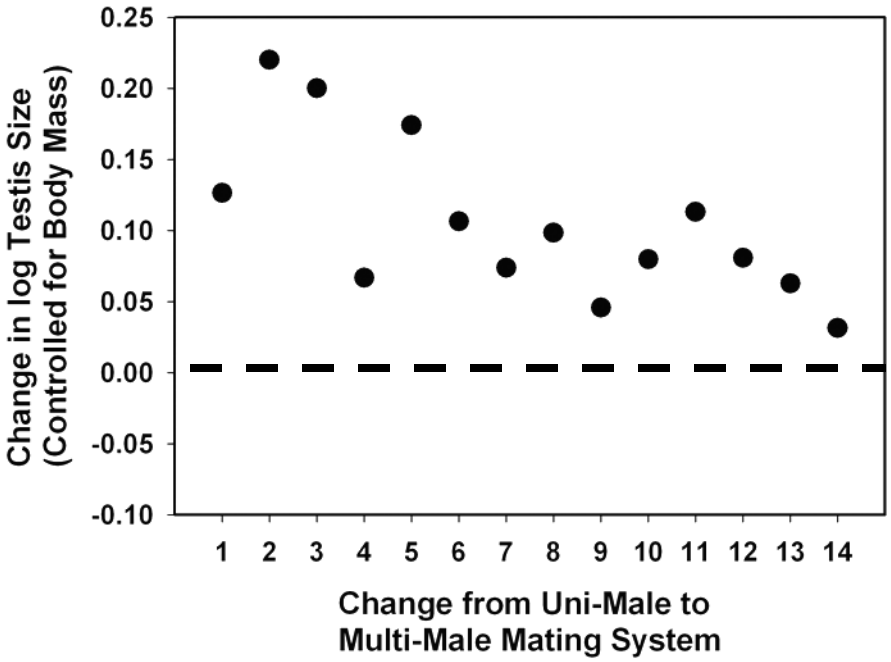
Figure 4. Independent contrasts ($N = 14$) of the evolutionary change in residual testis size (controlled for evolutionary change in body mass) vs. the evolutionary change from uni-male (monogamy or polygyny) to multi-male mating systems in primates. Contrasts were generated using the BRUNCH algorithm for categorical data in CAIC. Contrasts greater than zero result from the evolution of larger testis size associated with the evolution of multi-male mating systems (pg. 83).

FIGURES


a)
M06 (02)
M50 (04)
F08 M08 (02)
f07
F16 M20 (03)
F21 M39 (04)
f23
F06 (02)
f02 f09
M28 (03)
F10 M08 (03)
m24
F10 M29 (04)
f36
F01 M03 (02)
m04
F04 M07 (02)
m11 m14
F26 M40 (04)
f22 f28 m37 m49
M01 / M10 (02)
F14 M19 (03)
m17 m25
F14 M42 (04)
M51 (04)
N
100 m
b)
M26 (03)
F29 M35 (03-04)
f33 m43
m45 m48
F35 M31 (03-04)
f39
F38 (04)
f40 m46


Log Testis Size
Log Body Mass
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1
2
3
4
5
6
Tupaia tana
Tupaia longipes


0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
Change in Testis Size
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Change in Body Mass


0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
Change in log Testis Size
(Controlled for Body Mass)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Change from Uni-Male to
Multi-Male Mating System

CHAPTER III

Female-biased dispersal and gene flow in a behaviorally monogamous mammal, the large treeshrew (*Tupaia tana*)

ABSTRACT

Female-biased dispersal (FBD) is predicted to occur in monogamous species due to local resource competition among females, but this prediction has rarely been tested in mammals. I examined whether dispersal and gene flow are female-biased in two populations of the monogamous large treeshrew (*Tupaia tana*) in Sabah, Malaysia (NE Borneo). Genetic analyses provided strong evidence of FBD in this species. I found lower values for the mean corrected assignment index for adult females than for males using seven microsatellite loci, indicating that male individuals were more likely to be local residents. Adult female pairs were also less related than adult male pairs, as predicted for FBD. Furthermore, comparison of Bayesian coalescent-based estimates of migration rates using maternally and bi-parentally inherited genetic markers indicated that gene flow is female-biased in *T. tana*. The effective number of migrants between populations estimated from mitochondrial DNA sequence was more than three times higher than the number estimated using autosomal microsatellite markers. These results provide the strongest genetic support to date for the predicted association between monogamy and female-biased dispersal patterns in mammals. I argue that competition

among females for feeding territories creates a sexual asymmetry in the costs and benefits of dispersal in large treeshrews.

INTRODUCTION

Dispersal has important implications for population genetics and demography, as well as for our ability to predict population-level responses to environmental disturbance (Bowler & Benton 2005; Clobert et al. 2001). Sex biases in dispersal have often been observed, but the pattern differs among vertebrate taxa: female-biased dispersal (FBD) is typical in bird species, whereas males disperse and females are philopatric in most mammal species (Dobson 1982; Greenwood 1980; Clarke et al. 1997). Evolutionary models of sex-biased dispersal have drawn comparative support from the prevalence of different behavioral mating systems in mammals and birds. Over 90% of bird species live in male-female pairs (behavioral monogamy, Ligon 1999), whereas 95% or more of mammals exhibit polygamous mating systems (Clutton-Brock 1989). Theoretical approaches suggest that the same sexual asymmetries driving the evolution of mating systems should also influence the evolution of dispersal patterns (Perrin & Mazalov 1999).

Three non-mutually exclusive factors have been proposed to explain the association between mating systems and sex-biased dispersal: inbreeding avoidance, local mate competition, and local resource competition (Dobson 1982; Liberg & von Schantz 1985; Greenwood 1980; Favre et al. 1997). All three hypotheses predict male-biased dispersal in polygynous species, because male offspring may be in greater danger of mating with the care-giving parent (i.e. females often have longer tenure), may face more intense local competition for mates, or must compete for resources to attract females,

respectively. Asymmetries between males and females in the risk of inbreeding and mate competition are not predicted in monogamous species, because individuals of both sexes may have only one mate and the same number of offspring. However, intense local resource competition (LRC) may lead to FBD in monogamous species if females benefit from dispersal by gaining critical resources for reproduction.

Monogamy in mammals is highly associated with female use of exclusive territories (Brotherton & Komers 2003), primarily as a strategy to minimize feeding competition when predation and other factors do not favor group-living (Emlen & Oring 1977; Müller & Thalmann 2000; Reichard 2003). Reproduction in males is unlikely to be as severely limited by food resources as it is in females, and thus an asymmetry in the costs of philopatry may arise in monogamous species if females compete for access to feeding territories. LRC may also increase the rate of female aggression in multi-female groups, resulting in the expulsion of juvenile females by their mothers (e.g. primates, Pusey & Packer 1987; Dietz & Baker 1993). However, comparative data suggest that most juvenile dispersal is "voluntary" (Wolff 1993), because the costs of dispersal may be low when unoccupied areas are available to immigrants (Wolff 1994).

The prediction of FBD due to LRC in monogamous species has rarely been examined in mammals. Dobson's (1982) comparative study did not find an association between FBD and monogamy in mammals, but few data were (and still are) available for monogamous species. The combination of FBD and behavioral monogamy has been convincingly established for only four species using field data: two canids that form monogamous pairs within larger social groups (African wild dogs, *Lycaon pictus*, Girman et al. 1997; Frame & Frame 1976; Ethiopian wolves, *Canis simensis*, Sillero-Zubiri et al.

1996), and two monogamous rodents (California mice, *Peromyscus californicus*, Ribble 1992; American beavers, *Castor canadensis*, Sun et al. 2000). Unbiased measures of dispersal are difficult to obtain using traditional field techniques, especially for pair-living species that are widely dispersed in space and time (Koenig et al. 1996). Sex biases in dispersal may also be obscured by the geographic scale at which a given study is conducted (Ji et al. 2001; Fontanillas et al. 2004). However, genetic methods to detect both sex-biased dispersal and gene flow at varying spatial scales have recently become available that ameliorate these logistical problems (Goudet et al. 2002; Prugnolle & de Meeus 2002).

Several polygynous mammals have been studied using these genetic techniques, and as predicted either no sex bias (e.g. river otters, *Lontra canadensis*, Blundell et al. 2002) or male-biased dispersal (e.g. brush-tailed rock wallabies, *Petrogale penicillata*, Hazlitt et al. 2004; talar tuco-tucos, *Ctenomys talarum*, Cutrera et al. 2005) has been detected in most cases. However, genetic analyses have also revealed FBD multiple times in polygynous species (common wombats, *Vombatus ursinus*, Banks et al. 2002; bush hyraxes, *Heterohyrax brucei*, Gerlach & Hoeck 2001; kinkajous, *Potos flavus*, Kays et al. 2000; greater white-lined bats, *Saccopteryx bilineata*, McCracken 1984), especially among catarrhine primates (western gorillas, *Gorilla beringei*, Bradley et al. 2004; hamadryas baboons, *Papio hamadryas*, Hammond et al. 2006; humans, *Homo sapiens*, Seielstad et al. 1998). The only genetic studies conducted on behaviorally monogamous mammals have produced contrasting results. Male alpine marmots (*Marmota marmota*) disperse more often than females (Goossens et al. 2001), whereas FBD occurs in the greater white-toothed shrew (*Crocidura russula,* Favre et al. 1997). Additional genetic

studies of behaviorally monogamous mammals are clearly required to assess Greenwood's (1980) predicted association between monogamy and FBD.

In this study I used both bi-parentally and maternally inherited molecular markers to test the hypotheses of female-biased dispersal and gene flow in the large treeshrew, *Tupaia tana* (Mammalia, Scandentia) in Sabah, Malaysia (NE Borneo). Large treeshrews form dispersed, behaviorally monogamous pairs (*sensu* Reichard 2003) but forage solitarily, possibly as an adaptation to intraspecific foraging competition (Chapter 1). Variance in male reproductive success was not significantly different from female reproductive success in *T. tana*, suggesting that intra-sexual competition for resources may be equal to or greater than local mate competition (Chapter 2). These characteristics indicate that *T. tana* can provide an important test of Greenwood's (1980) LRC hypothesis for the evolution of sex-biased dispersal.

I tested the hypothesis of FBD in *T. tana* by comparing the genetic structure and patterns of relatedness among adult males and females at seven autosomal microsatellite loci. FBD is predicted to produce genotypes with lower population assignment probabilities and pairwise relatedness among adult (i.e. post-dispersal) females than among adult males in the population (Goudet et al. 2002; Prugnolle & de Meeus 2002). I also examined the hypothesis of female-biased gene flow in *T. tana* by comparing gene flow estimated from bi-parentally inherited microsatellite markers and a maternally inherited mitochondrial DNA (mtDNA) marker. Bayesian methods based on the coalescent (Beerli 2006) were used to estimate the exchange of migrants between *T. tana* populations in primary and selectively logged forests. If gene flow is female biased, then the migration rate for mtDNA should substantially exceed the migration rate for bi-

parentally inherited microsatellites. Inference of overall and sex-specific migration rates in this study also has conservation implications, given that forest fragmentation due to logging may disrupt the connectivity of wildlife populations in Borneo (e.g. orangutans, Goossens et al. 2005).

METHODS

Study sites and genetic sampling

Large treeshrews are small (200-250 g), diurnal, frugivore-insectivores that inhabit the lowland tropical rainforests of Borneo and Sumatra. I collected ear clips for genetic analyses from 54 *T. tana* individuals at two sites in Sabah, Malaysia (NE Borneo) from 2002-2004. The first site (N = 39 samples) was located in the Danum Valley Conservation Area (Danum, 4°58´N, 117°48´E) and consisted of undisturbed primary lowland rainforest. The other site (N = 15 samples) was located within the Malua Forest Reserve (5°5´N, 117°38´E), approximately 53 km from the primary forest site. This area was heavily logged in the early 1980's and has yet to recover the multiple closed canopies (typically 10 m and 20-30 m in height) and tall emergent trees (up to 70 m) that characterize lowland rainforests in SE Asia (Whitmore 1984). Fifteen of the 39 samples from primary forest and nine out of 15 samples from the logged forest site were obtained from juveniles. Only one juvenile was still present the year after its birth, and only two adults persisted for more than one year (Chapter 2). See Chapter 1 for full details of the study site and trapping methods.

I extracted genomic DNA from ear tissue samples using Qiagen DNeasy tissue extraction kits (Qiagen, Valencia, CA). Seven previously-described, unlinked

microsatellite loci named JS22, JS132, JS183, JS188, JS196, SKTg19, and SKTg22 were amplified using the PCR conditions in Appendix 1. Fluorescently-labeled alleles were separated on an Applied Biosystems 3100 DNA Analyzer and sized and scored using Genotyper 2.5 (Applied Biosystems, Foster City, California, USA). I also PCR-amplified a 602 bp segment of the mtDNA control region using the primers JMSTbel386 and JMSTbel1110 (see Chapter 2 for PCR conditions). I sequenced the forward strand of the PCR product using the BigDye Terminator 3.1 and a 3100 DNA Analyzer (Applied Biosystems). Sequences were edited and aligned using Sequencher 4.1.2 (Gene Codes, Ann Arbor, Michigan, USA) and Bioedit 7.0.4.1 (Hall 1999).

To examine differences in genetic variability between the primary and logged forest sites, I calculated the number of alleles and allelic richness at each microsatellite locus for each population using FSTAT v. 2.9.3.2 (Goudet 2001). I also used the log-likelihood G test of genotypic differentiation implemented in FSTAT (10,000 randomizations not assuming Hardy-Weinberg equilibrium, Goudet et al. 1996) to examine whether the two populations exhibited significantly different microsatellite allele frequencies. I investigated mtDNA sequence divergence between populations by calculating the number of fixed differences and shared mutations between populations, and the average nucleotide substitutions and number of net substitutions per site between populations (D_{xy} and D_a, respectively, with Jukes-Cantor correction, Nei 1987), using DNASP v. 4.2.4 (Rozas et al. 2003). I also conducted a permutation test (10,000 randomizations without alignment gaps) of genetic differentiation using the nearest-neighbor statistic (S_{nn}) implemented in DNASP. S_{nn} measures how often the most similar sequences in a data set ("nearest neighbors") are from the same population, and produces

a powerful test of genetic differentiation for sequence data in nearly all situations (Hudson 2000).

Tests of female-biased dispersal

I tested for FBD by comparing mean corrected assignment indices (mAI_c) between males and females using the "biased dispersal" module in FSTAT. One-sided *P* values were calculated using 10,000 randomizations. The assignment index is the probability that an individual's genotype occurred by chance in a population (Paetkau et al. 1995), and Favre et al. (1997) applied a correction that produces mean AI_c values of zero for each population. Negative AI_c values characterize individuals with genotypes that are less likely than average to occur in a population sample, and thus significantly lower mAI_c values for one sex (females, in this case) implies sex-biased dispersal. This index was chosen because both simulations and real data sets have indicated that this test has high power at detecting moderately intense biases in dispersal (Goudet et al. 2002; Mossman & Waser 1999). Adult genotypes were used for these analyses, because this test assumes post-dispersal sampling ($N = 9$ females and 15 males in primary forest, and $N = 4$ females and 4 males in logged forest). However, two putative juveniles from the logged site were included in the analysis because neither genetic nor behavioral parents could be assigned to them (f20 and m33, Chapter 2). Exclusion of these individuals did not substantially change the results.

I also tested the prediction that pairs of adult females were less related on average than pairs of adult males, because sex-biased dispersal is predicted to influence local relatedness structure among adults (e.g. Hazlitt et al. 2004; Banks et al. 2002). If female

T. tana disperse more often or farther than males, then fewer closely related pairs of females should occur in the sample. I calculated two estimators of pairwise relatedness, because the performance of different estimators varies depending on population composition (van de Casteele et al. 2001). Two method-of-moment regression estimators, Lynch and Ritland's *r* (Lynch & Ritland 1999) and Queller and Goodnight's *r* (Queller & Goodnight 1989), were calculated using the program MARK (Ritland & Travis 2004). Simulations indicate that the Lynch and Ritland estimator performs well for most population compositions (Thomas 2005). The Queller and Goodnight estimator is commonly used in studies of relatedness, and was included to facilitate comparison with previous analyses.

Pairwise relatedness estimates from the primary and logged forest sites were pooled to increase sample sizes, but relatedness was calculated only between pairs of individuals from the same site. For each different estimator, I tested whether mean female relatedness was lower than mean male relatedness using a two-sample randomization test (Manly 1991). Randomization tests were used because relatedness data were generated for dyads of individuals and thus do not represent independent observations. The one-sided *P* value for these tests was calculated by comparing the observed mean difference to the mean differences calculated from 10,000 randomizations of the same sets of relatedness estimates using POPTOOLS v. 2.6.6 (Hood 2005).

Tests of female-biased gene flow

If gene flow among large treeshrew populations is female-biased, then migration rates calculated for maternally inherited mtDNA should be higher than migration rates

calculated for bi-parentally inherited autosomal markers. To test this prediction, I used the Bayesian coalescence approach implemented in MIGRATE v. 2.1.3 (Beerli & Felsenstein 2001) to estimate the effective number of migrants exchanged per generation (N_em) between the primary and logged forest populations. Bayesian inference may be more accurate and efficient at sampling genealogy space than maximum likelihood approaches for many datasets (Beerli 2006). This method produces estimates of Θ ($4N_e\mu$, where μ = mutation rate) and M (m / μ) from microsatellite data, which when multiplied together equals $4N_em$. For mtDNA, this method estimates the effective number of migrants per generation as $2N_fm$ (N_f = effective population size of females). Assuming an equal sex ratio and equal male and female variance in reproductive success, N_f is approximately equivalent to N_e / 2 calculated from microsatellite data. Female-biased gene flow should thus result in higher estimated migration rates for mtDNA than for microsatellite data (Wright et al. 2005).

To estimate the effective number of migrants from microsatellite data, I ran 10 sequential iterations in MIGRATE using a stepwise mutation model with constant mutation rates, an exponential prior distribution (Θ distribution: minimum = 0.0, maximum = 0.1, mean = 0.01; M distribution: minimum = 0.000001, maximum = 1000, mean = 100), starting parameters based on F_{st} calculations, burn-in equaling 10,000 trees, five long chains sampling 2,000,000 genealogies, and an adaptive heating scheme (swapping interval = 1; four chains with start temperatures = 1, 1.2, 1.5 and 3). The same analysis was then repeated using the estimates of Θ and M obtained from the first analysis as starting parameters. In this second analysis, a search window for the exponential prior distribution was set according to the distribution of parameter estimates

from the first analysis ($\Delta = 0.03$ for Θ; $\Delta = 110$ for M). For the mtDNA dataset, I used the same analytical strategy with the F84 model of DNA sequence evolution instead of the stepwise microsatellite mutation model. However, I increased the number of sampled genealogies to 10,000,000 to achieve convergence, and used wider windows in the second run ($\Delta = 0.06$ for Θ; $\Delta = 250$ for M). These analyses produced values of ΘM ($4N_em$ and $2N_fm$ for microsatellites and mtDNA, respectively) estimated in each direction between the two populations along with their approximate 95% confidence intervals (0.025 and 0.975 posterior distribution values, Beerli & Felsenstein 2001). Following Wright et al. (2005), I then calculated the overall number of migrants per generation (N_em) by summing ΘM in each direction and dividing by four for microsatellites and two for mtDNA.

RESULTS

Genetic differentiation between primary and logged forest populations

Microsatellite allelic diversity was moderate in both *T. tana* populations, ranging from two to nine alleles (mean = 6.43) in the primary forest and from two to six alleles (mean = 4.0) in the logged forest (Table 1). Allelic richness, a measure of allelic diversity independent of sample size, showed a similar pattern. Genotypic differentiation between the two populations was highly significant overall ($P < 0.0001$), as well as for four out of the seven loci (JS183, JS188, SKTg19, and SKTg22; Table 1). There were zero fixed differences and 14 shared mutations between populations in the 602 bp mtDNA d-loop sequence. The average number of nucleotide substitutions per site between populations was $D_{xy} \pm \text{SD} = 0.026 \pm 0.007$, and the net substitutions per site was $D_a \pm \text{SD} = 0.0016 \pm$

0.006. In contrast to the microsatellite genotypes, genetic differentiation in the mtDNA sequence was not significant between the two populations (S_{nn} = 0.66, P = 0.16).

Female-biased dispersal

In agreement with predictions for FBD, I found significantly lower mAI_c for adult females than for adult males ($P < 0.05$, Table 2). Mean AI_c was negative for females (mean = -0.70) and positive for males (mean = 0.48), indicating that males are more likely to be resident individuals than females. Two method-of-moment estimators of relatedness, Lynch and Ritland's r (Figure 1) and Queller and Goodnight's r, indicated that pairs of adult females were significantly less related than pairs of adult males ($P <$ 0.05, Table 2).

Female-biased gene flow

Bayesian inference of migration rates produced an estimate for mtDNA of $2N_fm$ = 8.20 (95^{th} percentile = 1.64 – 24.56) from primary to logged forest and $2N_fm$ = 3.35 (95^{th} percentile = 0.07 – 13.46) from logged to primary forest. These two estimates correspond to an overall estimate of N_fm = 5.77. Assuming an equal sex ratio and low variance in male reproductive success, this value is equivalent to N_em = 11.54 effective migrants per generation exchanged between the two populations.

Microsatellite estimates of the effective number of migrants per generation were substantially less than mtDNA estimates. Bayesian inference produced an estimate across all seven loci of $4N_em$ = 12.26 (95^{th} percentile = 5.93 – 15.27) from primary to logged forest and $4N_em$ = 2.04 (95^{th} percentile = 1.05 – 3.40) from logged to primary

forest. These estimates correspond to an overall effective number of migrants per generation of N_em = 3.58, which is more than three times less than N_em estimated for mtDNA.

DISCUSSION

Multiple genetic analyses presented here provide evidence of FBD in large treeshrews. As predicted for FBD, adult females had significantly lower mean values than males for two different tests (mAI_c and pairwise relatedness). The genetic methods used in this study detect sex-biased dispersal only when adults have been nearly completely sampled and the sex bias is intense (e.g. 80:20 in simulated datasets, Goudet et al. 2002). A sex bias was detected for *T. tana* despite moderate sample sizes and genetic variability at seven microsatellite markers, suggesting that dispersal in *T. tana* is heavily female-biased. The magnitude of the sex difference in mAI_c for *T. tana* (1.18) was similar to significant values for two other small mammals in which sex-biased dispersal was also confirmed using trapping data (mean of 1.82 for two years due to FBD in greater white-toothed shrews, *C. russula*, Favre et al. 1997; 1.35 due to male-biased dispersal in white-footed mice, *Peromyscus leucopus*, Mossman & Waser 1999).

Evidence of FBD in *T. tana* was also provided by significantly lower relatedness values among adult females than among adult males for two pairwise measures of relatedness. Average male and female relatedness were negative for two method-of-moment regression estimators (Figure 1), but negative relatedness values are not unexpected given the high sampling variance of these estimators inherent in all but the largest data sets (e.g. > 40 loci, Lynch & Ritland 1999; Thomas 2005). Negative

pairwise relatedness results whenever one pair member exhibits the other's alleles at a frequency less than the estimated population frequency (Gardner & West 2004). Relatedness among females may thus be negative more often if immigrant females with genotypes that do not reflect overall population allele frequencies are present in the sample. A large proportion of related individuals (e.g. male relatives, as predicted if dispersal is female-biased) in the sample could also contribute to negative relatedness values for unrelated females. These methods do not distinguish between biases in the numbers of individuals of each sex dispersing vs. the distances dispersed. This study did not address whether males are philopatric, but male offspring born in one study period were typically not present on their natal territory in the following study period (Chapter 2). The differences in mAI_c and relatedness for *T. tana* were likely caused by females with uncommon genotypes that immigrated to the study site (i.e. a bias in the dispersal distance) rather than male philopatry.

The prediction of greater migration rates for maternally inherited markers than bi-parentally inherited markers was also supported by the results of this study. The overall number of migrants per generation estimated using mtDNA was more than three times higher than the microsatellite estimate. The substantially higher migration rate for mtDNA thus suggests that historical gene flow in large treeshrews has been highly female-biased. A recent simulation study indicated that migration rates and confidence intervals estimated from mtDNA using maximum likelihood coalescence techniques are often not accurate (Abdo et al. 2004). However, the Bayesian coalescence approach implemented in this study ameliorates these problems by achieving improved accuracy and more thorough genealogical sampling (Beerli 2006). The magnitude of the

difference in migration for mtDNA and microsatellite markers may be reduced if *T. tana* samples for this study violate the assumptions of an equal sex ratio and equal variance in male and female reproductive success. However, variance in reproductive success was not different between males and females, and the sex ratio of offspring was equal in these populations (Chapter 2), indicating that these assumptions are reasonable for *T. tana*.

This study is one of the first to find convincing genetic evidence of FBD and gene flow in a behaviorally monogamous mammal. Almost all genetic studies that have found evidence of FBD have been in polygynous species (e.g. Banks et al. 2002; Hammond et al. 2006). The only other genetic evidence of FBD in a monogamous species comes from a study on the temperate shrew *C. russula*, which also exhibited much lower mAI_c values among females than males (Favre et al. 1997). However, polygynous males with up to four female partners occur in *C. russula* (Bouteiller & Perrin 2000), and local resource competition between females has not been shown in this species (Favre et al. 1997). Behavioral pairs of *C. russula* also only persist for less than one breeding season, placing them at the short-term end of the continuum of pair duration in monogamous mammals (Reichard 2003). Results from large treeshrews may be more representative of the predicted association between monogamy and FBD due to LRC between females.

Greenwood (1980) predicted that monogamy would correlate with FBD because a sexual asymmetry in the costs of resource competition may favor the evolution of these two behavioral patterns. Foraging competition may have been a primary driver for the evolution of behavioral monogamy in large treeshrews (Chapter 1), and the same evolutionary pressures may act on dispersal in this species. *T. tana* live in behaviorally monogamous pairs, but forage solitarily and do not share sleeping sites. This dispersed

form of behavioral monogamy likely arose through a two-step evolutionary scenario: female avoidance and territoriality due to foraging competition, followed by male defense of a single female's territory to limit the number of other males feeding in the same area (Chapter 1, intersexual feeding competition hypothesis, Schülke 2005). Female body condition and reproductive output increase during supra-annual fruit masting events in Borneo, suggesting that fruit abundance is a key factor limiting reproduction in this species (Chapter 1, Emmons 2000). Large treeshrews also exhibit a unique, energetically-expensive absentee maternal care system that may limit their ability to produce young on poor-quality territories, or during periods of resource scarcity. Females nurse their litter of two pups for only a few minutes once every 48 hours, and must store large amounts of milk between nursing bouts (Emmons 2000; Martin 1966). These energetic limitations on reproduction are likely to produce intense competition between females for resources, leading to the observed territoriality and dispersal patterns in large treeshrews.

The costs and benefits influencing the evolution of behavioral monogamy appear to influence dispersal patterns in large treeshrews. The fitness benefits females gain from dispersal and the proximate factors influencing dispersal rates are fruitful areas for future research that could be addressed using provisioning experiments. Benefits males gain from philopatry, if any, also deserve closer examination. The results from this study also indicate that gene flow is ongoing between *T. tana* populations in primary forests and logged forests in Sabah, Malaysia. Southeast Asia has experienced greater rates of deforestation than other tropical regions (Sodhi et al. 2004), and Sabah is typical in that most of the valuable timber has already been extracted from its lowland rainforests

(Brookfield et al. 1995). Most vertebrate species are present after logging, but the connectivity of populations in primary and logged forests is not well understood (Grieser Johns 1997). I found significant genotypic differentiation at microsatellite loci between the primary and logged forest populations, but gene flow estimated for mtDNA suggests that female migration is sufficiently high to avoid rapid loss of genetic variation among large treeshrews in Sabah.

TABLES

Table 1. Number of alleles and allelic richness of seven microsatellite loci among large treeshrews from the primary forest ($N = 39$) and logged forest ($N = 15$) populations. *P* values correspond to 10,000 randomizations of log-likelihood *G* tests of population differentiation for each locus. The test of population differentiation over all loci was highly significant ($P < 0.0001$). See Appendix 1 for additional information on these loci (pg. 101).

	No. alleles			Allelic richness			
Locus	Primary	Logged	Total	Primary	Logged	Total	*P* value
JS22	9	5	10	6.33	4.87	6.18	0.11
JS132	2	2	2	2	2	2	0.64
JS183	12	6	12	8.74	5.93	8.68	0.02
JS188	6	6	8	4.86	5.93	5.86	<0.001
JS196	4	3	4	3.76	3.0	3.57	0.55
SKTg19	6	2	6	4.48	2.0	4.08	0.03
SKTg22	6	4	7	5.79	4	6.31	<0.0001
Mean	6.43	4.0	7.0	5.14	3.96	5.24	

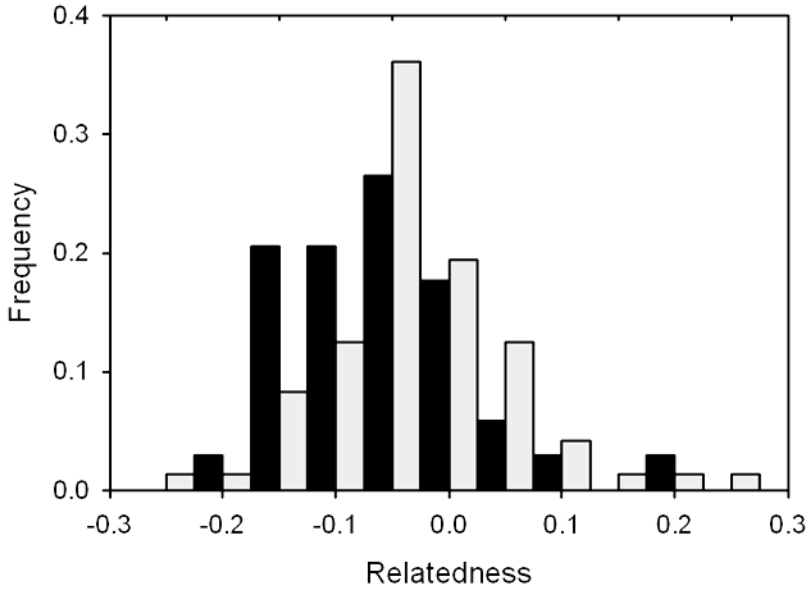
Table 2. Adult male and female means and tests of female-biased dispersal based on the corrected assignment index (AI_c) and two method-of-moment relatedness estimators. The *P* value for AI_c was based on 10,000 permutations in FSTAT (*N* = 19 males and 13 females), and *P* values for the relatedness estimators were based on two-sample randomization tests with 10,000 permutations (*N* = 72 pairwise *r* values for males and 34 for females; pg. 102).

Test	Adult male	Adult female	*P* value
AI_c	0.48	-0.70	0.05
Lynch-Ritland *r*	-0.05	-0.09	0.02
Queller-Goodnight *r*	-0.04	-0.09	0.05

FIGURE LEGENDS

Figure 1. Frequencies of pairwise relatedness values (Lynch & Ritland's *r*) for male (gray bars, $N = 72$) and female (black bars, $N = 34$) large treeshrews (pg. 104).

FIGURES



APPENDIX I

Isolation and characterization of polymorphic microsatellite loci in Bornean treeshrews (*Tupaia* spp.)

ABSTRACT

In this study I developed five microsatellite loci from an enriched genomic library constructed for the pygmy treeshrew (*Tupaia minor*), and adapted another two from a previous study on the common treeshrew (*Tupaia glis*), for use in studying mating and dispersal patterns in Bornean treeshrews. I screened 32 plain treeshrew (*Tupaia longipes*) and 54 large treeshrew (*Tupaia tana*) individuals at these loci. Polymorphism ranged from 2 to 13 alleles, and heterozygosity ranged from 0.29 to 0.88. These results indicate the general utility of these microsatellites for genetic analyses in other *Tupaia* spp.

MAIN TEXT

The treeshrews (Tupaiidae, Scandentia) are little-known but common mammalian inhabitants of the Indomalayan tropics. Their close phylogenetic affinity with primates (Sargis 2004) and relatively rare behavioral traits of absentee maternal care and behavioral monogamy (Emmons 2000) have recently attracted attention from researchers. Male-female treeshrew pairs defend joint territories against same-sex conspecifics, but individuals typically have access to extra-pair mates at the edges of their territorial boundaries, especially when ecological conditions are favorable (Chapter 1). I developed

five new polymorphic microsatellites from a genomic library created from pygmy treeshrew (*Tupaia minor*) DNA, and then adapted them for a study of mating and dispersal patterns in the large treeshrew (*Tupaia tana*) and plain treeshrew (*Tupaia longipes*) in Sabah, Malaysia (NE Borneo). I also designed six primer pairs for microsatellite loci previously sequenced from the common treeshrew (*Tupaia glis*, Srikwan et al. 2002), but only two produced polymorphic polymerase chain reaction (PCR) products from both *T. tana* and *T. longipes* DNA (SKTg19 and SKTg22, Table 1).

After digesting *T. minor* DNA with *Nhe*I, *Xmn*I, *Alu*I, and *Bam*HI (New England Biolabs (NEB)), I created a genomic library enriched for a dinucleotide repeat motif using the standard protocol of Hamilton *et al*. (1999). The enriched library was cloned into *Xba*I-digested P-bluescript SK+ plasmid vectors (Stratagene), and transformed into *Escherichia coli* Supercompetent cells (Stratagene) for cloning. Positive colonies were picked and heated for 10 min at 100 °C in 200 µl T.E (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). PCR of cloned DNA contained the following in a total volume of 30 µl: 50-100 ng DNA from each colony, 0.5 U Vent polymerase (NEB), 1X Thermopol buffer (NEB), 0.2 mM of each dNTP, and 8 µM of T3 and T7 primers. I used a PCR profile of 96 °C for 5 minutes followed by 30 cycles of 96 °C for 45 s, 51 °C for 1 min and 72 °C for 2 min. PCR products were run in 2% ethidium-bromide agarose gels to identify genomic DNA inserts of 70-1000 bp. I cleaned PCR products using the QIAquick PCR purification kit (Qiagen) and sequenced in one direction using the ABI BigDye ready reaction kit (Applied Biosystems). If clones contained microsatellites with at least seven dinucleotide repeats, then I sequenced them in the reverse direction and examined the resulting sequences in Sequencher 4.1 (Gene Codes).

I designed flanking primers for 18 sequences containing microsatellites using the Primer3 program (Rozen & Skaletsky 2000). I optimized primers for PCR amplification in *T. tana* and *T. longipes* using either a gradient or touchdown cycle on a PTC-200 Programmable Thermal Cycler (MJ Research). The annealing temperature in the touchdown program began at 65 °C and then decreased 0.5 °C per cycle to a final annealing temperature of 47.5 °C. I selected five primer pairs that showed evidence of length variation for use in our study of Bornean treeshrews, and the forward primer was fluorescently labeled with 6-FAM or HEX. I used the Qiagen DNEasy Tissue Kit (Qiagen) to extract DNA from ear-clips stored in 95% ethanol from 54 *T. tana* and 32 *T. longipes* individuals trapped at two different sites in Sabah. PCR were performed in 9 µl volumes containing 1 µl template DNA, 0.125 U *Taq* polymerase (Invitrogen), 1X PCR buffer (Invitrogen), 0.3 mM of each dNTP, 2.5 mM $MgCl_2$, and 0.55 µM of each primer.

Fluorescently-labeled alleles were separated on an Applied Biosystems 3100 DNA Analyzer and sized and scored using Genotyper 2.5 (Applied Biosystems). All seven primer pairs amplified PCR products in both *T. longipes* and *T. tana* (Table 1). Expected heterozygosity, tests of genotypic linkage disequilibrium, and deviations from Hardy-Weinberg equilibrium (HWE) were calculated using FSTAT (Goudet 2001). Mean observed heterozygosity ($H_O = 0.73$ for *T. longipes*, $H_O = 0.61$ for *T. tana*) was not significantly different from mean expected heterozygosity ($H_E = 0.74$ for *T. longipes*, $H_E = 0.58$ for *T. tana*) for either species. No loci were found to be in linkage disequilibrium for either species ($P > 0.05$). Loci were in HWE in both species except SKTg22 in *T. longipes* and JS183 in both species ($P < 0.01$). These two loci showed low observed heterozygosities relative to expected values. Polymorphism in these seven microsatellites

was generally moderate but ranged widely from 2 to 13 alleles. These results from two evolutionarily-divergent *Tupaia* spp. (Olson et al. 2005) indicate the general suitability of these primers for microsatellite analyses within this taxonomic group.

Table 1. Characteristics of microsatellite loci amplified from two species of Bornean treeshrews (F: Forward primer, R: Reverse primer; *Tl*: *Tupaia longipes*, *Tt*: *Tupaia tana*; pgs. 109-110)

Locus	Motif	Primer Sequence (5′ to 3′)	Size Range (bp)	No. of typed treeshrews	No. of alleles
JS22	$(GT)_3GC(GT)_{14}$	F: CAATGTCCTGGTGGTTATGG	*Tl*: 156-180	*Tl*: 32	*Tl*: 4
		R: GAAGTGGTCACTCTGCAATCC	*Tt*: 157-183	*Tt*: 48	*Tt*: 10
JS132	$(GT)_{15}$	F: GCCAACCACAGTTTGAGTCC	*Tl*: 219-259	*Tl*: 29	*Tl*: 5
		R: TCTTTATTGGGAAGGCATGG	*Tt*: 256-260	*Tt*: 48	*Tt*: 2
JS183	$(GT)_{15}$	F: GAAACAATAAGCCAGACTTCAGC	*Tl*: 115-153	*Tl*: 25	*Tl*: 12
		R: TCACGAGTAACCTACGATAGCC	*Tt*: 132-166	*Tt*: 54	*Tt*: 12
JS188	$(CA)_{13}$	F: ACACACACAAAACTCATTTTATCC	*Tl*: 170-200	*Tl*: 30	*Tl*: 11
		R: TCTACACGAATGTGCCAACC	*Tt*: 176-190	*Tt*: 51	*Tt*: 8
JS196	$(GT)_{19}$	F: ACCTCCTGGTGGCTTGC	*Tl*: 139-151	*Tl*: 32	*Tl*: 3
		R: TAATTGCAGGATGCTTCAGG	*Tt*: 227-233	*Tt*: 48	*Tt*: 4
SKTg19	$(CA)_7TAAA(CA)_8$	F: AAACCCCTCCCTAAAGGAAC	*Tl*: 167-197	*Tl*: 28	*Tl*: 11
		R: ACCCGCCCTATAGAAACCTC	*Tt*: 158-186	*Tt*: 53	*Tt*: 6
SKTg22	$(CA)_8A(CA)_{10}$	F: GAGTGCACTTGCCCTGTAAC	*Tl*: 133-187	*Tl*: 29	*Tl*: 13
		R: TCCTGAACCTGGTGGCTAAC	*Tt*: 160-176	*Tt*: 47	*Tt*: 7

Table 1. continued

H_O	H_E	Annealing Temp. (°C)	Accession No.
Tl: 0.88	*Tl*: 0.57	35 cycles;	DQ334277
Tt: 0.79	*Tt*: 0.60	65-0.5 / cycle	
Tl: 0.79	*Tl*: 0.66	54	DQ334278
Tt: 0.29	*Tt*: 0.37		
Tl: 0.73	*Tl*: 0.89	35 cycles;	DQ334279
Tt: 0.60	*Tt*: 0.79	65-0.7 / cycle	
Tl: 0.77	*Tl*: 0.86	57	DQ334280
Tt: 0.66	*Tt*: 0.63		
Tl: 0.62	*Tl*: 0.48	56	DQ334281
Tt: 0.54	*Tt*: 0.44		
Tl: 0.81	*Tl*: 0.82	51	AY064163
Tt: 0.62	*Tt*: 0.48		
Tl: 0.53	*Tl*: 0.90	57	AY064165
Tt: 0.78	*Tt*: 0.76		

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