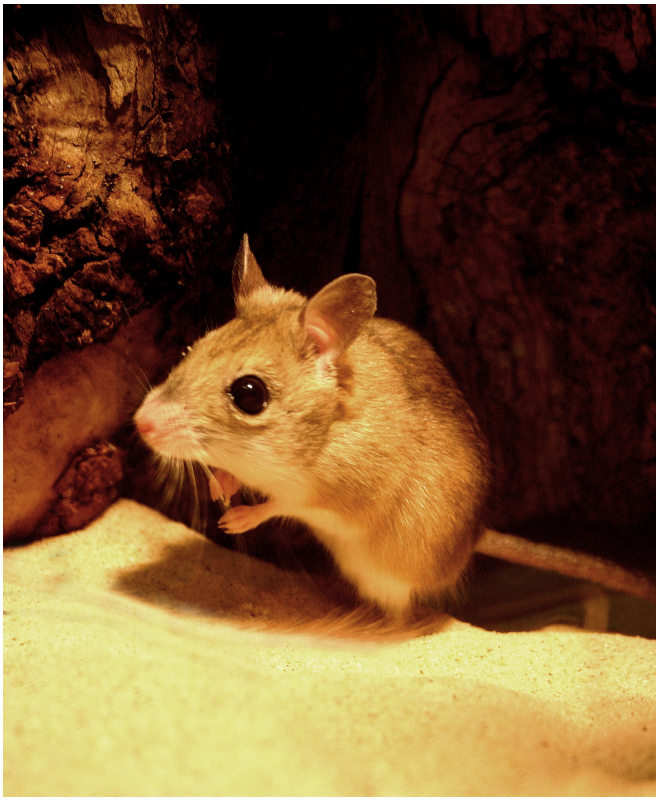
FEMALE-BIASED SEXUAL SIZE DIMORPHISM AND MATE CHOICE IN TARRKAWARRA (*Notomys alexis*)



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For the ginger tom,

Feisty

and Naughty Nancy

TABLE OF CONTENTS

Statement of Authorship		i
Statement of Contributions		iii
Note regarding formatting		v
Summary		vii
Acknowledgments		ix
Introduction		1
Chapter One:	Female-biased sexual size dimorphism in mammals: revisited, revised, reinvigorated	9
Chapter Two:	Growth to asymptote under different population densities in the female-biased sexually size dimorphic tarrkawarra (*Notomys alexis*)	59
Chapter Three:	Litter sex-ratio effects on female but not male growth in a small mammal with female-biased sexual size dimorphism	97
Chapter Four:	Can female mate-choice explain female-biased sexual size dimorphism in tarrkawarra (*Notomys alexis*)?	127
Chapter Five:	Male mate choice in tarrkawarra (*Notomys alexis*), a mammal with significantly female-biased sexual size dimorphism	165
Conclusion		205

STATEMENT OF AUTHORSHIP

I certify that the work in this thesis entitled "Female-biased sexual size dimorphism and mate choice in tarrkawarra (*Notomys alexis*)" has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by myself. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Ethics Review Committee, reference number: AEC2005018 on 6th November, 2007.

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STATEMENT OF CONTRIBUTIONS

Overall: Mariella Herberstein (my supervisor) provided her time for invaluable guidance, discussions on ideas and direction of research and writing expertise. Alan Taylor also provided guidance on statistical analyses throughout.

Chapter 1: Conception: 90% Compilation of statistics: 100% Writing: 80%.

Editing: Mark Eldridge (Australian Museum) provided invaluable comments on the manuscript.

Chapter 2: Conception: 90% Data collection: 100% Analysis: 60% Writing: 80%.

Data analyses: Alan Taylor greatly assisted with development of statistical process and provided guidance throughout analyses.

Chapter 3: Conception: 80% Data collection: 100% Analysis: 80% Writing: 80%.

Editing: Tobias Uller (Oxford University) provided invaluable comments on the theoretical development of arguments put forward in the manuscript.

Chapter 4: Conception: 100% Data collection: 90% Analysis: 80% Writing: 80%

Experimental design and preliminary analyses were discussed with Darren Burke. Data footage was blind scored from video by Melissa Holtz and Julia Howland.

Chapter 5: Conception: 100% Data collection: 90% Analysis: 80% Writing: 80%

Experimental design and preliminary analyses were discussed with Dr. Darren Burke. Data footage was blind scored from video by Julia Howland and Holly Maslowski.

NOTE REGARDING FORMATTING

Nearly every chapter in this thesis has been prepared for submission to a different journal. For consistency of presentation here, I have chosen to use a format that is generally consistent with that required by both Proceedings of the Royal Society (Chapter 3) and Animal Behaviour (Chapters 4 and 5). Submitted versions of the manuscripts prepared for Trends in Ecology and Evolution (Chapter 1) and Physiology and Behavior (Chapter 2) will reflect the formatting requirements of those two journals.

SUMMARY

Female-biased sexual size dimorphism (FSSD) is an unusual phenomenon in mammals, which are typically male-biased sexually size dimorphic (SSD). Mammalian FSSD is also poorly accounted for by theory and, in particular, by sexual selection theory (Chapter 1). In this thesis I demonstrate that tarrkawarra (*Notomys alexis* or spinifex hopping mouse), is an excellent model system with which to investigate mammalian FSSD for the following reasons: Firstly, captive tarrkawarra and some field populations demonstrate a high level of FSSD relative to other FSSD mammals. Secondly, sexual size parity in other tarrkawarra populations, implies intra-specific variation in the degree of FSSD expressed. Studies of intra-specific variation have some advantages over inter-specific studies. Thirdly, tarrkawarra are small, easy to maintain in captivity and common in their natural habitat (the Australian arid zone).

Based on the field data published, I determined that intra-specific variation in the degree of FSSD tracks the environmental fluctuations that increase tarrkawarra populations 40 to 100-fold between long 'bust' and short 'boom' periods (Chapter 2). Spatial distribution of resources and potential mates is usually a strong determinant of optimal mating system and flexibility in tarrkawarra social organisation therefore seems likely. Flexibility would also explain why attempts to categorise the mating system have never satisfactorily accounted for all observations of the animals' reproductive physiology and behaviour.

To test the flexibility of FSSD in tarrkawarra, I assessed developmental and environmental effects on the degree of FSSD in a captive tarrkawarra population. I found evidence for divergent male and female growth from birth, mitigated by both population density (Chapter 2) and by neonatal litter composition (Chapter 3). Specifically, litter sex-ratio effects on female growth and reproductive success suggest sex differentiated maternal investment. Litters sired by larger males were also found to have a higher sex ratio, suggesting that, despite low inter-male competition, sexual selection may still be important in this species via mating preferences.

Studies of first female (Chapter 4), then male mate choice (Chapter 5) support there being a role for sexual selection in the mating system of tarrkawarra. Both sexes demonstrated large-partner preferences, although male preferences reflected their own sexual experience and phenotype more than female quality. Consequently, these results cannot explain FSSD in tarrkawarra by themselves. Whilst there is now evidence of fecundity and mate choice selection for larger female size, selection on smaller male size has yet to be adequately described.

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INTRODUCTION

In a limited number of mammalian species females are larger than males. However, very few of these female-biased sexually size dimorphic (FSSD) species have been well studied and even fewer have been studied specifically in terms of sexual size dimorphism (SSD). Mammalian-based theory regards these species as anomalous and theoretical understanding is weakened by making few concessions to account for them (Lindenfors et al. 2007). The apparent complacency with extant mammalian SSD theory, and paucity of empirical data on FSSD species, is surprising because, from a non taxon-specific perspective, it is pronounced male-biased SSD that is anomalous and theoretically problematic. As exceptions to the rule, FSSD mammals should be of central interest in unravelling this dilemma.

The role of sexual selection in maintaining mammalian FSSD has been particularly overlooked in the literature. This can be traced to a widely held assumption that FSSD mammals fail to observe the mammalian norms of competitive males and/or reproductively constrained females (Ralls 1976; Isaac 2005). However, male-male competition is documented in a small number of FSSD mammals. Furthermore, in the majority of cases, there is no *a priori* reason why large-female fecundity selection should be greater in FSSD than it is in non-FSSD mammals (Chapter 1). With genetic determination of paternity, ever-increasing numbers of mammals are being re-branded from strictly polygynous or monogamous to polyandrous and promiscuous. This is true of species from the across the full range of SSD patterns, making the under-lying tenets

of mammalian SSD increasingly questionable even for non-FSSD species (McEachern et al. 2009).

My thesis aims to draw attention to the theoretical inconsistencies described above and to begin to redress the paucity of research on sexual selection in FSSD mammals. In particular I aim to position tarrkawarra (*Notomys alexis* or spinifex hopping mouse) as a model FSSD species for the purpose of such research. In doing so it was important to first review the current literature and theory with respect to mammalian female biased sexual size dimorphism (Chapter 1) and to document the extent and plasticity of FSSD in this species (Chapter 2). I then investigated some fitness consequences of size for females (Chapter 3). Thereafter I aimed to determine the direct mating preferences of both sexes (Chapters 4 and 5), these being highly indicative of the direction and strength of sexual selection in many species.

Chapter 1 updates the only previous review of mammalian FSSD that I am aware of - a provisional and speculative list of FSSD mammals published to inaugurate the field (Ralls 1976). Over 30 years later, it is still the principal source cited in synthesis of the literature. To redress this, I collated empirical citations for FSSD mammals that were not included on Ralls's original list and for mammals subsequently disqualified from it. These citations are predominantly species-specific studies on aspects of ecology and behaviour other than FSSD. I also identified certain cladistic-biases in study species and re-assessed Rall's dismissal of sexual selection as a mechanism maintaining FSSD in mammals. I argue that this dismissal no longer reflects our broadened interpretation of sexual selection and the mechanisms by which it is achieved. I also suggest that extant data make phylogenetically independent conclusions

about the broader function of mammalian FSSD problematic and that empirical data are needed on a much broader range of species if theory is to advance further.

Chapter 2 introduces tarrkawarra, a widely distributed FSSD species that is easily maintained in captivity as well as non-threatened in its' natural habitat. These factors, as well a comprehensive body of extant research on the reproductive physiology of the species, make it an ideal model species. The purpose of this paper is two-fold: Firstly it aims to clarify the uniformity (or otherwise) of FSSD in tarrkawarra, based on field data suggesting that fluctuating population densities affect size dimorphism. I addressed this by comparing the male and female weights of young tarrkawarra weaned into one of two conditions that were aimed at replicating high and low population density conditions in the field. Secondly this paper aims to determine developmental contributions to FSSD that occur in this species, with a focus on the different implications of early versus late sexual weight differentiation (Blanckenhorn 2005). To do this I tracked the growth trajectories of male versus female offspring from birth to their asymptotic weights at full adulthood (2.5 years).

Chapter 3 explores ways in which size-dependent differences in female fitness might contribute to the maintenance of FSSD. I approached this in two ways: Firstly I assessed whether increases in breeding female and male weights were associated with increases in the weight, number or litter sex ratio of the pups they produced. Secondly I assessed evidence for sex-differentiated maternal investment by tarrkawarra dams according to litter sex ratio. Sex-differentiated maternal investment is theoretically expected to reflect the different fitness potential of female relative to male offspring and typically favours the production of large sons over daughters or small sons in male-

biased SSD species. This increases parental fitness because the sole hypothetical constraint on male fitness (mating success, but see Chapters 4 and 5) is diminished for large sons relative to that of small males. This offsets the limitations on the reproductive output of daughters due to 'time out' whilst gestating (Trivers & Willard 1973). In contrast to male-biased SSD mammals, studies of FSSD species usually conclude that size-dependent female fecundity selection must underlie the sexual size differentiation observed. If this is the case then large females in FSSD species, such as tarrkawarra, should (a) have a fecundity advantage over smaller females and (b) should invest preferentially in the production of large daughters. I tested these two hypotheses in tarrkawarra by regressing breeding female weights against the size and sex ratio of litters they produced and by analysing pup growth by sex in litters of different sex ratios.

Chapter 4 investigates whether pre-copulatory mate choice in female tarrkawarra contributes to the maintenance of FSSD. Pre-copulatory mate choice is the most direct form of sexual selection although there are, of course, other forms. Research on testicular volume and sperm production in tarrkawarra indicates that sperm competition is unlikely (Breed & Ford 2007) and mammals are not thought to be equipped for cryptic mate choice. Pre-copulatory mate choice seemed possible based on observations made when I was establishing the breeding colony for my research: In particular females aggressively repelled certain males they were paired with, resulting in male injuries and mortalities if I did not intervene. Female tarrkawarra are known to be aggressive towards unfamiliar males (Stanley 1971; Happold 1976) however, pairings were sometimes unsuccessful even after reasonable familiarisation periods. I therefore assessed female mate choice in relation to male familiarity (unfamiliar or familiarised

using an extensive olfactory and proprioceptive process) and also in relation to male size (smaller or larger than an alternative male). Ethical standards regarding surplus pups precluded use of choice tests that terminate with copulation, so I gauged mating motivation from changes in female responding to males when sexually receptive compared with when they were sexually non-receptive. I also compared the response of sexually inexperienced (virgin) females with sexually experienced (unsuccessfully mated) females.

Chapter 5 investigated pre-copulatory male mate choice in tarrkawarra on the basis that a male preference for mating with larger females might contribute to FSSD in a sperm limited species. Additionally recent theoretical and empirical papers have suggested that the Batesian assumptions that underlie our neglect of the study of male mate-choice should be questioned (Green & Madjidian 2011; Bro-Jørgensen 2010). This seems especially appropriate in the context of tarrkawarra because it is (a) a species with sex-reversals in both size dimorphism and in inter-sexual aggression (female-female aggression is greater than that between males (Happold 1976; Stanley 1971) and (b) a species in which sperm limitation and female-male aggression probably impose male mating costs that are at odds with typical assumptions of male and female sex roles. To assess male mate-choice I used a similar procedure to that used to gauge female mate choice (Chapter 4). I assessed male association preferences based on both relative size and familiarity, related to whether females were sexually receptive or not. Additionally I compared the responses of males that were sexually experienced (mated) with those that were inexperienced (virgin) and responses based on whether males were the smaller or the larger of their same-sex home cage pair. This final variable was

included to see if small males were in any way able to compensate for the large-male preference observed for female tarrkawarra in Chapter 4.

Each of my chapters has been prepared as a self-contained manuscript for publication in the journal designated on the title page. As such there is some overlap in chapter introductions and species descriptions. A final synthesis of the thesis chapters puts my findings into context within the field of mammalian FSSD research and discusses the potential of tarrkawarra as a model system for future research.

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Chapter 1

Female-biased sexual size dimorphism in mammals: revisited, revised, reinvigorated

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In preparation for submission to *Trends in Ecology & Evolution*

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PREFACE

Rall's (1976) seminal list of female-biased sexually size dimorphic (FSSD) mammals is ripe for review. Additional FSSD species have been identified since, others confirmed as non-FSSD. I argue that theoretical development of FSSD has been limited since Ralls due to taxonomic pseudo-replication. Data exist for multiple species, but within a limited number of clades. Clade-based studies increasingly indicate relationships between behavioural plasticity, sexual selection and patterns of sexual size dimorphism (SSD) but phylogenetically unconstrained testing of these relationships is now needed. I also advocate exploring FSSD as a function of the habitat groupings evident in Ralls (aerial, aquatic or terrestrial). This is increasingly viable as data on FSSD in small, terrestrial mammals amass to a level comparable with those for aerial and aquatic species.

WHY STUDY FSSD IN MAMMALS?

Female-biased sexual size dimorphism (FSSD) is typical of insects and most invertebrates (Blanckenhorn 2000) yet male-biased sexual size dimorphism (SSD) is the overwhelming norm in mammals (Lindenfors et al. 2007). Neither selection for large males through male-male competition, nor selection for small female size through tradeoffs between growth and age at first reproduction, are complete explanations of this phenomenon for at least four reasons: Firstly, sexual selection on large male size and growth/reproduction tradeoffs are by no means exclusively mammalian scenarios (e.g. giant weta Kelly et al. 2008), nor are they universally absent in FSSD mammals (e.g. horseshoe bats Rossiter et al. 2006; and yellow-pine chipmunks Schulte-Hostedde & Millar 2002); Secondly, female size constraints are usually outweighed by fecundity benefits in non-mammalian taxa and fecundity benefits from large female size also exist in many mammals (e.g. Bornholdt et al. 2008); Thirdly, positive correlations between male size and reproductive success are inconsistent, or absent, in mammals where small males use alternative mating strategies (e.g. Heckel & von Helversen 2002; Ortega et al. 2008) or where small male size enhances performance in scramble polygyny (Bondrup-Nielsen & Ims 1990): Fourthly, FSSD does occur, albeit infrequently, in 12 of the 29 mammalian Orders (Ralls 1976).

Ralls (1976) listed over 200 mammalian species for which FSSD had been verified, reported or speculatively noted. In these mammals, selection for large male size is assumed to be absent, or weakened relative to selection for large female size. More specific, integrated hypotheses to explain the full range of mammalian SSD are lacking (Isaac 2005). FSSD mammals are key to generating such hypotheses because constraints on male

size (which must also exist in male-biased SSD species) are more easily identified in the absence of strong counter selection for large male size. Likewise, if FSSD occurs because putative constraints on mammalian female size are relaxed, pinpointing such constraints would be facilitated by comparison of male-biased SSD with FSSD species. Yet, despite their value to theoretical development, empirical data on FSSD systems since Ralls (1976) has been published only erratically.

The purpose of this paper is to evaluate research on FSSD in mammals since Ralls (1976) and to describe some interesting cases that have been verified or added to the list since. I will also make a case for a) considering more species that indicate convergent evolution of FSSD; b) specifically evaluating mammalian FSSD in the absence or presence of 'special case' physiological constraints (such as flight) and; c) exploring and controlling for the effects of behavioural plasticity and environmental instability on FSSD.

RALLS LEGACY – THEN AND NOW

Ralls (1976) listed over 200 possible FSSD species and this has frequently been misinterpreted as indicating widespread FSSD amongst mammals (e.g. Fokidis & Risch 2008; Rossiter et. al. 2006; Lammers et. al. 2001). However, for approximately 80% of the species she listed, Ralls carefully noted that the FSSD classification was tentative - based on anything from limited but convincing data to anecdote. Many species on this provisional list have since been confirmed as having sexual size parity or male-biased SSD (e.g. aardwolfs van Jaarsveld et al. 1995). When I omitted species for which less than convincing data were available at the time Ralls' list was published, only 135 species - that

is 2.5% of all mammalian species - were then verified or highly likely to be FSSD (Table 1).

FSSD in multiple species within a single taxonomic group may or may not represent multiple evolutionary events (Cardini & O'Higgins 2004). It is therefore important to note that of the 2.5% of mammals indicated as FSSD by Ralls (1976), nearly half come from just two Orders, the first being the volant chiropterans (bats), the second the aquatic cetaceans (whales and dolphins). Seals bolster the aquatic presence in Ralls list, and are confined to a single phylogenetic grouping (Phocidae), within the Order Carnivora (Table 1). Since Ralls paper, FSSD has been established as typical of the specious chiropterans (Lindenfors et al. 2007) which constitute 21% of all mammal species. However, the very different selective forces associated with life on the wing make bats non-representative of the predominantly terrestrial mammalian class. The same may be true of selection on size in aquatic environments (Box 1).

Considering terrestrial FSSD species, ungulates constitute 23% of Ralls original list, although ungulates represent just 4% percent of all mammals. The ungulates listed by Ralls (1976) are also conspicuously all in a single phylogenetic grouping (Bovidae - antelope, duikers and dikdiks). Other terrestrial species are scattered across Orders in no clear pattern. For example, the twelve rodent species listed are from six distantly related families. It is particularly remarkable how few rodents were included in Rall's list as they are disproportionately represented in mammalian taxonomy at approximately 42% of the 5418 mammalian species (Simmons & Geisler 1998).

Box 1. The problem with flying (and swimming)

Flying produces peculiar issues in mammals. Unlike birds, female mammals gestate, lactate and often transport young. Females in many bat species carry their young until they are near adult weight (Kunz & Hood 2000). Volant and glissant female mammals (i.e. bats, flying squirrels and other gliders) must therefore accommodate additional 'wing-loading' demands (Dietz & Kalko 2007). Consequently, in many of the FSSD bat species, selection on female wingspan may outweigh constraints on female size (Hayssen & Kunz 1996; Myers 1978). For example, the species-specific degree of FSSD increases with average litter size in Microchiroptera (Greenwood & Wheeler 1985). Conversely smaller size confers greater aerial agility in bats and thus may be selected for in males because it can improve harem recruitment and defense (Knörnschild et al. 2010). Similarly, if sexual segregation makes aerial foraging agility more important for male than for female success, this may drive FSSD in volants. For example, in several insectivorous FSSD bats small size and aerial agility equip males to feed exclusively on flying insects. Females feed predominantly on ground or foliage-based prey. This requires less aerial maneuverability, conserves energy and supports the reproductive fitness advantages of larger female size (Cryan et al. 2000; Senior et al. 2005).

Various avian FSSD systems resemble volant FSSD mammals in having selection on smaller male size for aerial hunt agility (Raihani et al. 2006) or sex-differentiated foraging patterns (Krüger 2005). However, the potential for paternal investment in birds (incubation and offspring provisioning) and the lack of wing-loading during gestation, makes the evolution of FSSD in birds far less complicated than it is in volant mammals.

Selection on size in marine mammals may also be regulated by very different factors than those that exist for terrestrial mammals. Constraints on female weight in aquatic environments are lessened by buoyancy and may be outweighed by thermal and swimming efficiency advantages of large size (Boyd 2002; Vogel 2006). The fully aquatic cetaceans have a high incidence of FSSD at 26-27% of species. However FSSD is only common in pinniped species that mate at sea, thus minimising their time ashore in the breeding season (Table 1).

The list of FSSD terrestrial mammals includes a number of species with highly arboreal lifestyles (e.g. squirrels). Selection on FSSD in these species may have some parallels to that observed in some bat species (Fokidis & Risch 2008a). Flying squirrels, for example, carry their young and are physiologically adapted for gliding (Fokidis & Risch 2008b), as, to some extent, are a number of primate species (sifakas and galagos Feduccia 1993) that did not quite meet criteria for inclusion on the list. In other squirrels, the more arboreal the species, the longer and the more FSSD the tail (Hayssen 2008). Squirrel tails aid in balance and leap span and this pattern may therefore indicate fecundity selection on females.

Strictly terrestrial mammals, such as antelope, most carnivores and non-arboreal rodents, have fewer or less obvious constraints on body size than species with arboreal or aquatic lifestyles (Gasc 2001). The selective forces on body size in volant and aquatic mammals are therefore specific enough to their modes of locomotion that they cannot be simply generalised to strictly terrestrial mammals.

Updating Ralls (1976) list (see figures in parentheses in Table 1), there are now approximately 213 verified FSSD or highly likely FSSD species. Increased numbers notwithstanding, this still indicates FSSD amongst just 4% of extant mammals. In some cases updated totals represent changes in species included rather than or as well as additions. For example, based on mass, three further primate species now appear on the FSSD list, whilst the single species previously listed (common marmoset) is now regarded as non-FSSD (Weckerly 1998). For many of the species added to the FSSD list (especially bats and some rodent groups), the degree of FSSD was reported to be statistically significant. Alternatively, where only ratios were published, I set an inclusion criterion of FSSD $\geq$ 1.05 (female/male body size measurement) with a minimum sample of 30 individuals of each sex (except aquatics – see Table 1 description). For comparison, there were only four terrestrial mammals in Rall's original list where females were at least 5% larger than males. I opted for such a conservative inclusion criterion because I found that reviews of SSD are often compromised by the use of secondary citations and/or unreliable sources, especially in the case of primates (see Smith & Jungers 1997 for a comprehensive discussion of this point). In determining which species to include I accepted FSSD ratios based on either length or mass. Although there is debate as to the relative worth of the two measures (Boonstra et al. 1993), the two figures scale remarkably well in the case of at least some species (Hayssen 2008), and body mass data are usually available when length data are not.

Aquatics and volants still account for nearly half of all FSSD mammals listed herein, but terrestrial species have nearly doubled (from 61 to 113) across a range of Orders, especially Rodentia (Table 1). In contrast to volants and aquatics, the FSSD terrestrial species continue to be relatively phylogenetically isolated - with a few

exceptions. FSSD is regarded as typical (on average) of the non-specious Order Lagomorpha (Lindenfors et al. 2007). It is also common amongst Bovidae (27% of Bovidae species), and ubiquitous amongst one other ungulate family (Tragulidae). In the former case, there has been little change in the species count since Ralls, but this may reflect the fact that ungulate SSD data are scant and hard to interpret.

Rodent numbers listed herein have nearly quadrupled since Ralls (1976), from 12 to 43, reflecting further study of vole, squirrel and chipmunk families in particular (Levenson 1990; Ostfeld & Heske 1993; Lammers et al. 2001; Gattermann et al. 2002; Mann & Towe 2003). I have probably underestimated the true extent of FSSD in this Order, at least in the case of squirrels. Ratios of SSD indicate that females of many squirrel species are substantially larger than males, but sample sizes were too small to meet my inclusion criteria. In particular, the addition of just six flying and tree squirrel species (Sciurinae) to the FSSD list is probably overly conservative. FSSD is likely widespread amongst the arboreal and volant squirrels (Box 1), particularly if tail length is considered in concert with (and / or independently of) head-body length (Hayssen 2008).

The six FSSD vole species, identified since Ralls (1976), fill out the spectrum of SSD forms, from male-biased, through to female-biased, in the specious Arvicolinae clade (151 species). This makes voles an excellent system for SSD studies with phlyogenetic contrasts (Bondrup-Nielsen & Ims 1990). A range of SSD is also now known to occur in the chipmunk family. In this case, however, FSSD is the norm rather than the exception, with 15 of 25 congeners having larger females than males and the remainder having sexual size parity or statistically non-significant male-biased SSD (Levenson, 1990).

Table 1. Summary and update of Ralls' list of FSSD species

Group/ Order*	Common name	Species Count	Source(s)
VOLANTS			
Chiroptera	Evening and vesper bats	23 (44)	(Bornholdt et al. 2008)
	Leaf-nosed bats	11 (10)	(Ortega et al. 2008)
	Sac-winged & sheath-tailed bats	6 (6)	
	Horseshoe bats	4 (8)	(Dietz et al. 2006)
	Other microchiropteans	3 (3)	
	Megachiropteran bats	2 (4)	(Rahman & Abdullah 2010; Storz et al. 2001)
		49 (75)	
AQUATICS			
Cetaceans	Baleen whales	9 (13)	(Aguilar & Lockyer 1987; Horwood 1986; Ralls & Mesnick 2008; Spitz et al. 2000)
	Beaked whales	2 (4)	(MacLeod 2006)
	Dolphins & porpoises	6 (7)	(Ralls & Mesnick 2008)
Sirenia	Dugong	1 (1)	
Carnivora: Pinnipedia	Earless seals	7 (8)	(Boness et al. 2002; Lindenfors et al. 2002; Mellish et al. 2011; Mesnick & Ralls 2008; Ralls & Mesnick 2008)
		25 (33)	
TERRESTRIALS			
Artiodactyla: Bovidae & Tragulidae	Duikers, Dikdiks & Antelope	29 (30)	(Brotherton & Manser 1997; Estes 1992; Goldspink et al. 2002)
	Chevrotains	1 (8)	(Jarman 1983 citing K. Ralls, pers comm)
	Other ungulates	0 (1)	(Weckerly 1998)
		30 (39)	
Lagomorpha	Cottontail rabbits	4 (6)	(Davis & Roth 2008)
	Hares & jackrabbits	3 (3)	(Crawford et al. 2010)
	Misc other rabbits	2 (1)	(Webb 1993)
		9 (10)	
Rodentia	Deer & grasshopper mice	5 (5)	(Johnson & Armstrong 1987; Mann & Towe 2003; Williams et al. 1985)
	Lemmings & voles	0 (7)	(Bondrup-Nielsen & Ims 1990)
	Other Myomorpha	3 (6)	(Breed 1983; Gattermann et al. 2002; Whitaker 1963; Wrigley 1972)
	Tree and flying squirrels	1 (6)	(Hayssen 2008; Schulte-Hostedde 2007)
	Chipmunks	0 (15)	(Gannon et al. 1993; Gannon & Forbes 1995; Hayssen 2008; Levenson 1990)
	Other Sciuromorpha	1 (2)	(Hayssen 2008)
	Hystricognath rodents	2 (3)	(Lammers et al. 2001; Sachser et al. 1999)
		12 (43)	
Carnivora	Mongooses	2 (2)	
	Hyenas	2 (1)	(van Jaarsveld et al. 1995)
		4 (3)	
Primates	Lemurids & others	**1 (3)**	(Dunham & Rudolf 2009; Gordon 2006; Smith & Cheverud 2002)
Other Orders	Cuscus, senjis and others	**5 (5)**	
TOTALS		**135 (211)**	

**Taxonomic classification follows Wilson & Reeder (2005) except for Chiroptera classification which follows Simmons and Geisler (1998).*
Full list of species will be made available in electronic supplementary material

Table 1. Frequency of species listed in Ralls (1976) as either "1, female larger than males" or "2, females probably larger than males". Sources given are for additions, removals and confirmations and parentheses contain updated frequencies. Minimum inclusion criterion for volant and terrestrial additions, removals and confirmations was female/male mass or length ≥ 1.05 with minimum ns of 30 per sex. Mass and body length data for most aquatic mammals are scarce so aquatic additions are necessarily based on similarly high (but estimated) degrees of FSSD reported. "Other Orders" are Diprotodontia ('Marsupialia' in Ralls 1976), Macroscelidea, Erinaceomorpha and Soricomorpha ('Insectivora' in Ralls 1976).

Box 2. FSSD and Rensch's Rule

Female-biased sexually size dimorphic (FSSD) species are particularly useful in the study of SSD (male or female-biased) because they allow us to test the full reach of Rensch's rule. Rensch's rule formalises an observed pattern that, amongst closely related species, the relative size difference between the sexes tends to increase with the overall body size of the species (hyperallometry) (Abouheif & Fairbairn 1997). Rensch's rule is observed in many but not all mammalian taxa, often depending on whether it is tested at the level of genus, family or order (Lindenfors et al. 2007). For example, it is observed in mammals overall, as well as the Orders Primates (Lindenfors et al. 2002) and Diprotodontia (kangaroos, possums, koalas etc.), but in no other Orders yet analysed (Lindenfors et al. 2007). However, Rensch's rule is also observed in Bovinae ungulates (antelopes, cattle, gazelles etc.) overall, in Caprinae (goats, sheep etc.) as a sub-group within this family (Polák & Frynta 2009, 2010) and in the rabbit genus Sylvilagus (Davis & Roth 2008).

Rensch's rule theoretically applies to both male and female-biased SSD. However, data fit best when the taxon analysed is predominantly under sexual selection for large male size (Webb & Freckleton 2007). In turn, the conventional explanation for hyperallometry (e.g. Fairbairn 2007) accommodates polygynous species very well, suggesting that selection on male body size simultaneously increases female size via a shared genome and the fitness consequences of bearing increasingly larger sons. This continues until female body size optimum is compromised. Thereafter sex-specific mechanisms influencing body size become adaptive, and female size is disproportionately constrained relative to male size (Fairbairn 1997). Groups where FSSD species predominate, however, often defy

Rensch's rule (confirmed when the slope of male to female body size exceeds one). Specifically, FSSD is predicted amongst only the smallest species of any given taxonomic grouping (Webb & Freckleton 2007). Implicitly these species are also expected to be only weakly, if at all, polygynous. These conditions are not always met (Table 2) and data fit lessens as FSSD species are added to analyses. For example, the Orders Chiroptera, Cetacea and Lagomorpha (rabbits) contravene Rensch's rule (Lindenfors et al. 2007) and pinnipeds also contravene it as a family (Abouheif & Fairbairn 1997). That not withstanding, Rensch's rule is occasionally observed at the intra-specific level, between populations (e.g. Crowther et al. 2003; Storz et al. 2001 in FSSD and non-FSSD species respectively). Likewise intra-specific effects that conform to Bergmanns rule (clinal variation in body size) and population density effects are well known. These effects may elucidate the proximal ecological factors that trigger the evolution of SSD (Roitberg 2007). Such phenotypic variation from a single genotype, interacting with different local conditions, becomes the target of selection, potentiating genotypic, sex-specific differentiation (Cornwallis & Uller 2010; Ledevin et al. 2010).

The power of Rensch's rule to predict relative mammalian body size is increasingly undermined by amassing empirical data on FSSD species. Ultimately, Rensch's rule may only consistently hold for animals with male-biased SSD and/or some degree of polygyny. As such it becomes a circular, self-fulfilling premise, at least at the macro-evolutionary level (Webb & Freckleton 2007). However, there is still much value in formulating observed patterns, such as Rensch's rule, to interpret intra-specific variation and the evolutionary consequences of proximate factors.

Box 3. Tarrkawarra (*Notomys alexis* or spinifex hopping mouse)

Tarrkawarra is an addition to Ralls's (1976) FSSD list that is particularly promising as a model terrestrial species for the following reasons:

1. Tarrkawarra demonstrate strong FSSD: Tarrkawarra exhibit FSSD of 1.19-1.33 (female/male non-reproductive adult body mass) depending on the population sampled (Smith et al. 1972; Breed 1983). Most FSSD species range from 1.01 to 1.05 and only four terrestrial mammals listed in Table 1 exceed this value.

2. Tarrkawarra are relatively phylogenetically unconstrained: In the specious Muridae family (720 species), only tarrkawarra and one distantly related South American mouse are FSSD. Tarrkawarra congeners (four extant, five extinct) closely resemble it but exhibit sexual size parity. However, the genus is morphologically very distinct from other endemic Australian rodents (Breed 1985).

3. Tarrkawarra demonstrate limited, if any, male-male competition: Male-male aggression is very rare (Stanley 1971; Happold 1976) and sperm competition is also unlikely as males have a very low testes:body mass (Breed & Taylor 2000) and remarkably inefficient spermatozoa production (Peirce & Breed 2001). Tarrkawarra males also produce sub-functional, if any, post-copulatory plug (Breed 1990), relative to other rodents. Even posterior glands that secrete pheromones eliciting inter-male aggression are diminutive in this rodent (Breed & Ford 2007). In most other FSSD systems, positive selection on male size does occur to some extent (Table 2). However, selection on male

'agility' may stabilise male size selection in many FSSD species. In tarrkawarra, stabilising sexual selection on male phenotypes seems highly unlikely given the absence of male-male competition. 'Big mother' fecundity selection notwithstanding, 'small male' selection may therefore parsimoniously explain FSSD in this species.

4. Tarrkawarra show high levels of intra-specific variation in FSSD: Behaviours consistent with both pair-bonding (e.g. paternal care of offspring) and promiscuity (multiple mating of both sexes) have been observed (Breed & Adams 1992; Bauer & Breed 2008) and patterns of immigration and recruitment between field sites suggest flexible social organisation (Dickman et al. 2010). This corresponds with fluctuating male weights, which are heaviest at high-population densities while mean female weights are similar during low and high densities (Breed 1992; Bauer & Breed 2008), resulting in a variable degree of FSSD. Social flexibility, and corresponding changes in the degree of SSD, is observed in many animals in response to environmental fluctuations (Isaac 2005). However, population-density is often used as a proxy for environmental status, with food availability assumed to retard growth only once populations approach and exceed maximum environmental carrying capacity (Bonenfant et al. 2002; Hewison et al. 2002). In the case of tarrkawarra, drops in population-density precede declines in food availability (Dickman et al. 2010). Tarrkawarra therefore also represent an excellent opportunity to decouple the effects of population density and food scarcity

The remaining terrestrial species on the updated Ralls (1976) list of FSSD mammals, comprise four small insectivores, three carnivores, the spotted cuscus and a dozen or so distantly related rodents. Relative to bats, ungulates and marine mammals (also hyenas), FSSD in these terrestrial mammals is, as yet, largely uninvestigated. Given their relative taxonomic isolation, FSSD in these species is likely to result from isolated evolutionary events. Such instances of independent evolutionary events may be key to understanding when and why FSSD becomes adaptive.

Ralls (1976) explicitly encouraged the study of SSD and FSSD within closely related mammalian groups and the taxonomic skews in her list implicitly facilitated this (Table 1). The result has been study-species biases, in particular towards bats and ungulates. Both groups span the continuum from male-biased to female-biased SSD. Consequently comparisons between FSSD and non-FSSD species of bats and ungulates can be easily made and related to differences in their mating systems, the latitude of their habitats, their foraging niches and so forth (Ruckstuhl & Neuhaus 2000). However, repeated analyses within these closely related groups results in phylogenetically non-independent replication (Diniz-Filho et al. 2010), limiting conclusions about mammals more generally. Research on both FSSD and SSD in bats and ungulates has resulted in a number of working hypotheses about the evolution of FSSD, particularly as a response to environmental variability (Table 2). Phylogenetically isolated terrestrial FSSD species represent an untapped opportunity to test these and other hypotheses (Table 2).

FUNCTIONAL EXPLANATIONS FOR FSSD

In Table 2, I have consolidated the hypotheses invoked to explain FSSD in mammals into very broad categories (relative to those available for FSSD in other taxa). The field is currently too small and poorly developed for more detailed and mechanistic elaboration. However, as research on mammalian FSSD continues to develop, we are likely to be to able to break these broad categories into more fine-scale explanations.

Current theory regards SSD (be it female or male-biased) as an epiphenomenon because body size is not usually a sex-linked trait and sex-specific selection is costly (Blanckenhorn 2005; Connallon et al. 2010). Sexual selection on body size in one sex affects the opposite sex simultaneously producing sexual size parity unless (or until – see Rensch's rule, Box 2) there is counter selection on body size in the other sex (the 'differential equilibrium model' of SSD: Andersson 1994). Accordingly, explanatory mechanisms in Table 2 are not mutually exclusive. One or more selective process per sex is usually required to explain why optimal body size becomes sex-differentiated (Lindenfors et al. 2007). In particular 'big mother' effects (Table 2(a), point 1) – whereby larger female size is correlated with increased fecundity and/or offspring viability - cannot explain FSSD in mammals alone. 'Big mother' effects are widespread and well described (Blanckenhorn 2005; but see Gaillard et al. 2000; Lindenfors et al. 2007). There are many different mechanisms associated with big mother hypotheses (e.g. increases in offspring birth mass, offspring number, prenatal and post natal growth rates as well as increases in the mothers' lactation capacity and duration and any number of other factors that directly affect her reproductive success) I have grouped these together in Table 2 to avoid

excessive replication of previous reviews. Big mother effects are, however, unremarkable in the literature. This is, at least in part, because they can be attributed to the mother's condition as much as her size in most, if not all, cases, and there is a prevailing correlation between size and condition (Blanckenhorn 2000). Apart from wing loading in bats, big mother effects observed in FSSD mammals do not indicate stronger female size selection than that seen in many non-FSSD mammals. In male-biased SSD mammals, selection on large male size clearly counters big mother effects. In FSSD species conditions that amplify selection on female size and/or condition must coincide with relaxed or negative selection on large male size.

Large, Competitive Females

Ralls (1976) considered that big mother effects combined with female-female competition were likely to account for much FSSD, presupposing that females of greater size will be more competitive. Wherever female-female competition involves direct physical aggression, this seems reasonable, however, relative to the sustained bouts of mating-related aggression seen amongst males, taxing physical altercations between females are rare. Direct female-female altercations do occur in species where females defend territories and a few FSSD mammals where females compete to secure exclusive access to optimal foraging and nesting sites are listed in Table 2(a), point 2. However, as with most explanations for FSSD in mammals, at least as many non-FSSD examples of female-female competition exist. Additionally even amongst FSSD species, territorial exclusion is not always achieved by direct physical confrontations. For instance, in several of the monogamous FSSD ungulate species, territories are passively maintained via scent markings of both sexes (Roberts & Dunbar 2000; Stockley & Bro-Jørgensen 2010).

Without direct interactions between female competitors, this form of territoriality seems unlikely to be contributing to the maintenance of FSSD.

An alternative form of female-female competition alluded to by Ralls (1976) relates to jostling for dominant positions in social hierarchies. These scenarios are even more problematic for explaining mammalian FSSD than female territoriality, at least because there are fewer reported instances of this phenomenon. It is also seldom the case that female social status is straightforwardly predicted by size. Attaining higher social status and preferential access to resources (especially food) may well allow females and their offspring to gain greater mass, and thus maintain their social status via physical means, compared to subordinates. Nevertheless, the basis of a female's social status is often not her size per se. For example, females in a number of non-FSSD ungulate and primate species, as well as hyenas, inherit their mother's status (Dusek et al. 2007). Reproductive suppression by dominant females may also be achieved via hormonal cues rather than, or in addition to, physical subordination (eg. a number of the naked mole rat species Faulkes & Bennett 2001). Whilst aggressive behaviour may be a mechanism to reinforce dominance, the singular importance of size in establishing female dominance is far from clear.

However, recent non-FSSD ungulate studies have re-invigorated research on female-female competition and highlighted that scenarios in which males rather than females may become a limiting resource are all too easily overlooked (Bebie & McElligott 2006; Bro-Jørgensen 2007; Powell 2008; Robinson & Kruuk 2007). Where males are a limiting resource, female-female competition is likely to escalate. In at least one case, the African topi, this apparently results in physical altercations (female displacement of

competitors during mating in order to initiate mating themselves), in which larger females may have an advantage. Preferred males of the African topi may be a limited resource because they become sperm depleted due to high-frequency mating within a restricted period of female oestrous synchronicity (Bro-Jørgensen 2002, 2007). Whilst the interference topi females direct at mating pairs has not been documented in an FSSD species as yet, the value of exploring non-conventional explanations for atypical sex roles, such as sperm limitation, is clear and has been understated to date (Kokko et al. 2005; Wedell et al. 2002).

Small, Agile Males

Male-male contest competition and defense polygyny (Table 2(b), point 2) are often assumed to be lessened or absent in FSSD species. The flaw in this argument is that, unless selection on female size is substantially stronger than in most mammals, relaxed selection on male size should only produce sexual size parity, not FSSD. As previously mentioned, apart from increased wing-loading in bats, all identified or postulated benefits of large female size for FSSD mammals are equally applicable in any number of non-FSSD mammals.

The fact that relaxed selection on male size is clearly not the case for many FSSD species, is at least as problematic as the absence of 'special case' selection on increased female size. For example, direct physical male-male altercations over mating (charging, striking, swiping, etc.) occur in several aquatic FSSD species (Table 2). Larger males are likely to win these fights, and relatively low testes:body mass indicates sperm competition is unlikely to act as compensatory mechanism for smaller males (Spitz et al. 2002). It is possible that the relative value of large size over agility is different in aquatic male-male

competition (Box 1), especially where aquatic males do not engage in direct physical combat. However, selection on increased male size for combat is a well-established mechanism maintaining extremely male-biased SSD in many pinnipeds (Lindenfors et al. 2002). Selection on agility may reverse that pattern in certain species. For example, Mediterranean monk and Weddell seals engage in acrobatic aquatic displays that probably serve to defend territory and mates (Mesnick & Ralls 2008) and may be more efficiently performed by smaller males. Differences in mating success related to acrobatic displays, locomotor speed, maneuverability, and even distance stamina, metabolic efficiency and endurance in resource limited situations, have all been described as 'agility' effects that may select for smaller male size (Kelly et al. 2008; Ralls & Mesnick 2008; Voigt et al. 2005; Weckerly 1998).

The abundance of aquatic examples under point 2 (Table 2(b)) is probably largely coincidental, simply reflecting the patchy distribution of research on FSSD mammals. Substantially more empirical data are required before conclusions can be made about how FSSD fits into models of SSD in aquatic mammals. However, in a number of the FSSD bat species selection for male agility can counteract selection for large male size via male-male competition. Resource defence polygyny is adaptive for males in most bat species as females typically cluster in maternal roosts or harems (Behr et al. 2009; Kunz & Hood 2000; Nagy et al. 2007). Harem defense is performed aerially via male chasing or hover guarding, both of which require flying agility. Hovering may also serve as a courtship display in some species (Knörnschild et al. 2010). Conversely, defense of clustered females may be replaced by scramble competition when females are more spatially dispersed (Bondrup-Nielsen & Ims 1990). In these cases male agility (or, perhaps rather, 'gracility') may be adaptive in terms of tradeoffs between size, locomotor performance,

energy conservation and starvation resistance during long distance mate-searching (Blanckenhorn 2005).

Agility as a selective advantage for FSSD males in terrestrial mammals is more convincing when it is not related to male-male combat or resource defense polygyny mammals (as, for example, in voles, see above). Scramble competition is likely to favour the most mobile or wide-ranging males and locomotor agility may be greatest in smaller males (Table 2(b), point 3). Accordingly in a number of terrestrial FSSD species mean mass and/or length is female-biased but hind leg size is male-biased (Davis & Roth 2008; Kelly et al. 2008; Wrigley 1972). Relatively less mass but more leverage to mobilise suggests that mobility may be under greater selection in males than in females in some species. Again detailed data on a great many more FSSD species are required before this observation can be incorporated into a broader pattern.

Amassing further data on relationships between range size and male agility in FSSD species should also prove particularly instructive. This is because both between and within-species variation in population density, distribution, and home range richness (irrespective of how this variation is brought about), is common, producing a natural 'experiment'. Under scramble competition, in an FSSD system, less spatially scattered females should reduce selection on agility and consequently also the degree of FSSD, as males are released from selection on small size. The relationship between SSD and female spatial distribution has been demonstrated in the microtine family, which includes several FSSD species (Bondrup-Nielsen & Ims 1990). In some polygynous male-biased SSD mammals, between-population differences in population density also correspond with differences in the degree of SSD observed (Bonenfant et al. 2002; Hewison et al. 2002).

Any change in the degree of FSSD between populations, in response to fluctuations in environmental conditions, is also likely to entail changes in optimal mating strategy. For example, in the FSSD oribi antelope, male mating strategies change from territorial defence and monogamy to female defense polygyny when supplementary feed is made available to wild populations (Brashares & Arcese 2002), although it is not known whether this also affects the degree of FSSD.

Agile male selection has become a very popular 'panacea' explanation for FSSD in mammals, probably at least in part because contest, display and scramble agility selection have all been well documented in FSSD bird and invertebrate species (Greenwood & Wheeler 1985; Kelly et al. 2008; Krüger 2005; McDonald et al. 2005a, b; Rittschof 2010). However, whilst some parallels exist in FSSD bats, agility selection in most other mammals is, as yet, a matter of speculation (Blanckenhorn 2000) and many more studies of the fitness consequences of agility in FSSD mammals are needed.

When selection on large male size is relaxed, it has been suggested that FSSD may result because male fitness is increased by early maturation at the expense of increased size (Table 2(b), point 1). This is a reversal of the scenario observed in many male-biased SSD species, whereby sexual size differentiation results from (or is inflated by) earlier female maturation and shorter duration growth relative to males (Lindenfors et al. 2002). In the case of male-biased SSD, males delay reproduction until they are large enough to defend multiple mates or otherwise secure a share of mating opportunities that are available predominantly only to larger males. In the case of polygynandrous FSSD species male fitness may be more effectively increased by an early entry into scramble competition and bypassing of some of the costs of becoming and staying large (Blanckenhorn 2000). This is

an elegant premise, however I have found no reports of FSSD mammals in which males mature earlier than females. Notwithstanding this, the early male maturation hypothesis might apply at a broader phylogenetic level. In this case it would be expected that the greater the degree of FSSD between species, the earlier the age of first male reproduction. FSSD, despite sexual parity at maturation, would also have to be explained by sexually differentiated early growth rates rather than duration and juvenile SSD is generally regarded as rare (Blanckenhorn 2005). Unfortunately, at this stage there are insufficient data on growth rates, duration and ontogeny in general in FSSD mammals for the early male maturation theory to be tested.

Mate Choice (Both Sexes)

Mate choice is arguably the most direct form of sexual selection studied but it has barely been explored in FSSD mammals (Table 2(a), point 3 and Table 2(b), point 4). Along with study species bias, this may be part of Rall's legacy to the field (e.g. Edelman & Koprowski 2006). Ralls (1976) suggested there was little to no role for sexual selection (presumably on either sex) in the FSSD phenomenon and that research on it would be non-productive. However, sexual selection is a) often cryptic and b) a pervasive aspect of most mammalian lives. At the very least, the relative impact of sexual selection on body size optima, needs to be evaluated in FSSD species. Additionally, changes in the strength of sexual selection operating on a species or population, probably have a key role in the evolution of SSD (Box 2). Increasing evidence suggests that the degree of both female and male-biased SSD varies depending on local conditions (Hewison et al. 2002; Johannesen & Andreassen 2008). Optimal mating system may also vary with the degree of SSD and population density (Kokko et al. 2005; Rittschof 2010; Schradin & Pillay 2005). The result

is that the strength of sexual selection in operation, between populations, is by no means a fixed variable.

At this stage, the paucity of data on sexual selection in FSSD mammals makes it impossible to analyse the extent to which between-population differences in optimal mating systems correlate with the degree of FSSD. Yet mating preferences are relatively easily assessed and provide an indicator of the strength and direction of sexual selection in a given population. For instance it is well established that female preferences for familiar over unfamiliar mates are indicative of pair bonding and monogamy as opposed to more polygynous systems where unfamiliar males are often preferred (Hurst et al. 2008). In short, the value of research on mate choice, and flexibility in mate choice, in FSSD species should be reassessed.

Sexual Segregation

Finally, sexual segregation hypotheses, as means to explain FSSD should be considered. These hypotheses do not fit the Table 2 format because they specifically predict simultaneous selection on both large female and small male size. Broadly, sexual segregation describes the phenomenon of sexually-differentiated habitat use. I have borrowed the term sexual segregation from the bat and ungulate literature (for reviews see Main 2008; Ruckstuhl & Neuhaus 2000; Ruckstuhl & Neuhaus 2002 (ungulates); Senior et al. 2005 (bats)) but it includes related theories such as 'sex differentiated foraging', 'niche specialisation' and 'the intra-sexual competition hypothesis' (Blanckenhorn 2005; Krüger 2005). This family of hypotheses posit that diminished intra-sexual conflict over foraging, nesting sites, or other ecological resources, will increase the fitness of both sexes and, in the case of SSD, allow one sex to therefore become larger. The phenomenon is

Table 2 (a). Functional explanations for FSSD citing a large female advantage

Large Female Advantage	Direct/Indirect Support in FSSD Mammals	Contradictory Findings
1. 'Big Mother' hypotheses …higher fecundity and/or offspring viability.	BATS (direct): Female fecundity increases with wing-loading in vesper and Pteropodidae bat species (Myers 1978; Storz et al. 2001). WHALES (indirect): Mother and calf length are positively correlated in humpback whales (Pack et al. 2009). RABBITS (indirect): female body size is positively correlated with litter size in common hares and jack-rabbits (Virgos et al. 2006). SCIURID RODENTS (indirect): female body size is positively correlated with pups produced in yellow-pine chipmunks and southern flying-squirrels (Fokidis et al. 2007; Schulte-Hostedde et al. 2002). OTHER RODENTS (indirect): female body size is positively correlated with offspring birth mass and reproductive success in bank voles (Mappes et al. 2004; Oksanen et al. 2007).	BATS (direct): female body size is independent of wing span in Western long-eared myotis (Solick & Barclay 2006). RABBITS (direct): Neither female body size nor FSSD correlate with annual reproductive output across the cottontail family (Davis & Roth 2008).
2. Female-female competition and female dominance …greater access to resources that indirectly increase fitness (food, territory, mates) including via suppression of access of other females (dominance).	BATS (indirect): Female territoriality occurs in Daubenton's bats (Dietz & Kalko 2007). UNGULATES (indirect): Female-female aggression, dominance, territorial defense and/or reproductive suppression occur in several species (Roberts & Dunbar 2000; Stockley & Bro-Jørgensen 2010). SCIURID RODENTS (indirect): Female size correlates with dominance over both sexes in Townsend chipmunks (Edelman & Koprowski 2006). Female size correlates with home range richness and litter size in southern flying squirrels (Fokidis et al. 2007). OTHER RODENTS (indirect): Female size correlates with dominance over both sexes in golden hamsters and dominant females reproductively suppress smaller females (Huck et al. 1988).	RABBITS (indirect): Female size does not correlate with dominance in this Order. Other forms of female-female competition are rare (Davis & Roth 2008). RODENTS (indirect): Female size does not correlate with home range size or litter size in bank voles (Koskela et al. 1997). CARNIVORES (indirect): Female size does not correlate with dominance or reproductive success in hyenas (Engh et al. 2002).
3. Male mate choice …male preference for larger females.	WHALES (indirect): female length is positively correlated with number of 'escort' males in humpback whales (Pack et al. 2009).	

Table 2 (b). Functional explanations for FSSD citing a small male advantage

Small Male Advantage	Direct/Indirect Support in FSSD Mammals	Contradictory Findings
1. Mature small, father early	NONE: Hypothetical reduced development time (and mortality risk) prior to starting reproduction yet to be empirically validated.	NONE: However, SSD (female or male biased) is seldom seen prior to sexual maturity (Blanckenhorn 2000) and predation, parasitism and starvation-susceptibility are often higher for smaller than larger animals (Blanckenhorn 2005).
2. Male-male competition and female defence polygyny …greater success in male-male competition for mates where agility or speed is required.	BATS (indirect): Male size is inversely related to hover duration (a harem defence behaviour), harem size and mating success in greater sac-winged bats (Knörnschild et al. ; Voigt et al. 2005). SEALS (speculative): Mediterranean monk and Weddell seal males practice aquatic territory/female defence. This may be most efficiently achieved by the smallest, most agile, individuals (Harcourt et al. 2007; Ralls & Mesnick 2008).	WHALES (indirect): In right, humpback and several of the beaked whales, males engage in direct physical competition for females (Mesnick & Ralls 2008; Spitz et al. 2002). DUGONGS (indirect): Males engage in direct physical competition for females (Ralls & Mesnick 2008). SEALS (indirect): Crabeater seals fight for and mate guard females ashore (Mesnick & Ralls 2008).
3. Agile male and scramble competition …speed, efficiency and endurance in foraging and mate searching or patrolling, which increases fitness and/or reduces mortality.	DOLPHINS AND DUGONGS (speculative): Males patrol large home ranges overlapping with several females (Ralls & Mesnick 2008). RABBITS (speculative): Males patrol large home ranges, overlapping with several females (FSSD species only) (Davis & Roth 2008). RODENTS (direct): Male relative to female size decreases with increasing distance between female territories in FSSD microtine voles species (Bondrup-Nielsen & Ims 1990). CARNIVORES (speculative): Male hyenas must disperse to breed and then tenaciously accompany pre-estrus females, often without feeding, for long periods (Engh et al. 2002).	SCIURID RODENTS (indirect): no small male advantage in reproductive success or running speed in yellow-pine chipmunks (Schulte-Hostedde & Millar, 2002).
4. Female mate choice …female preference for smaller males.	BATS (indirect): Hover duration and mating success correlate negatively with male body mass in greater sac-winged bats (Voigt et al. 2008). SEALS (speculative): Acrobatic courtship displays are observed in male Weddell seals in the presence of estrus females (Ralls & Mesnick 2008).	WHALES (indirect): Female humpback whales preferentially associate and mate with longer males (Pack et al. 2009). CARNIVORES (indirect): No female preference for small males in hyenas (Engh et al. 2002).

documented in many mammals, but has been criticised as an explanation for SSD because sexually-differentiated habitat richness may simply maintain and reinforce, rather than cause, SSD (Blanckenhorn 2005). Additionally sexual segregation occurs not only in SSDspecies but also in some with sexual size parity (Blanckenhorn 2005).

SUMMARY

Evidence of widespread FSSD amongst mammals has not been forthcoming in the 35 years since Ralls first comprehensively documented the phenomenon. Species in which females are the larger sex must still be regarded as uncharacteristic but important conundrums to be explained. Whilst there has been progress to this end within a few distinct clades (notably in bats), taxonomic biases have resulted in extensive pseudo-replication. The result is that synthesis of mammalian data on FSSD, to inform theory at

Table 2. (previous page) Hypotheses postulated to explain FSSD in mammals via selection for larger females (a) or selection for smaller males (b). Examples are all drawn from research on or including FSSD species. Nature of support for or against hypotheses is given in parentheses. 'Direct' indicates a specific test of the hypothesis that analyses variation between different populations of a species or variation between related species of a genus, family or Order. 'Indirect' indicates other findings that are pertinent to the hypothesis but which do not specifically analyse FSSD as a variable. 'Speculative' indicates a hypothetical relationship between observations of relevant variables.

a more general level, is still not feasible. In particular, there is a shortage of data on terrestrial FSSD species to effectively complement our sophisticated knowledge of male-biased SSD in (predominantly terrestrial) mammals.

The extant working hypotheses explaining mammalian FSSD are patchy and underdeveloped. In order to confirm or repudiate theory for what drives the evolution of FSSD in mammals (and SSD more generally), comprehensive studies of species in which FSSD is phylogenetically independent are now required. Study species should be targeted from previously under represented, terrestrial dwelling groups, with a particular emphasis on those that can be compared with well documented, non-FSSD species that have similar habitats, diets, and ecological niches.

Finally, there is great potential to overcome the issue of phylogenetic comparison altogether by studying model FSSD systems where intra-species variation in the degree of FSSD is known to occur (e.g. Box 3). These models should illuminate the important interaction of environmental and phenotypic plasticity in the evolution of sex-differentiated traits (Cornwallis & Uller 2010), especially in settings where ecological variables can be systematically manipulated. Plasticity in inter-population responses to environmental change is arguably the most promising tool we currently have to systematically resolve the evolutionary puzzle that is FSSD in mammals.

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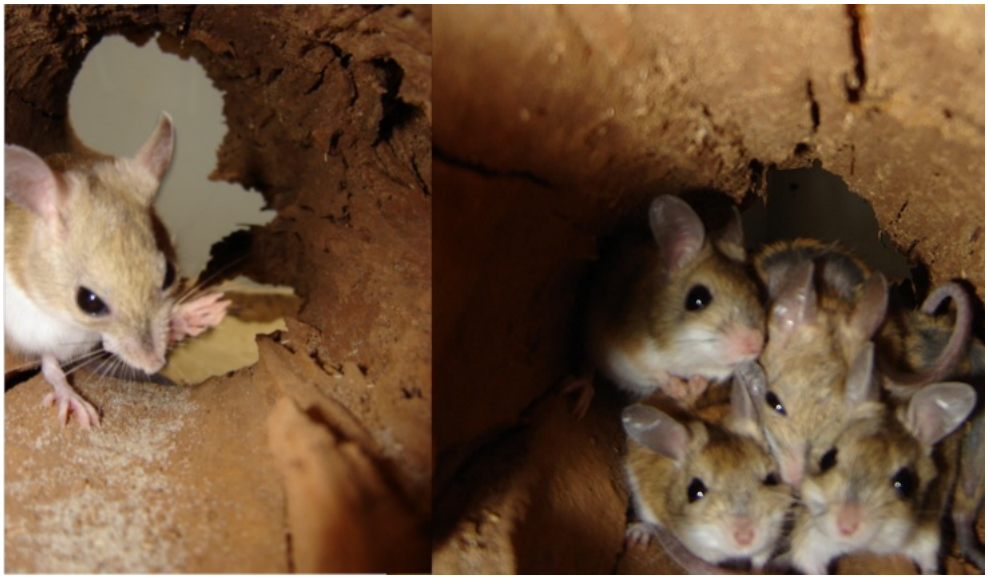
Chapter 2

Growth to asymptote under different population densities in the female-biased sexually size dimorphic tarrkawarra (*Notomys alexis*)

In preparation for submission to *Physiology and Behavior*



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ABSTRACT

Our understanding of mammalian sexual size dimorphism (SSD), and its relationship with mating systems, is hindered by limited empirical data on species where females are the larger sex. Captive tarrkawarra (*Notomys alexis* or spinifex hopping mice), an arid zone rodent, are female-biased sexually size dimorphic (FSSD) in early life. However, field data suggest FSSD may vary with population age and density. The aim of this study was to assess how sex differences in size are established in this species and the relative contributions of growth rate, growth duration and population density to the degree of FSSD in fully-grown, age-matched tarrkawarra cohorts. We assessed sex differences in neonate weights and growth of weanlings in high or low-density housing conditions (25 days to six months of age). We then tracked growth of these animals through to asymptotic weight (the weight at which the slope of the entire remaining growth curve approaches zero) in standardised housing (six months onwards). Tarrkawarra females in this sample were significantly heavier than males from birth, despite longer gestation periods for male-biased litters. Neonatal weights predicted male growth trajectories, whilst weaning weights predicted female trajectories. Post-weaning growth increased the degree of FSSD in low-density housed animals whilst high-density housed animals initially attained sexual size parity. Growth to asymptotic weight was achieved at 18 and 24 months for males and females respectively, and reduced between-condition differences in FSSD under the housing density manipulation. Flexibility of FSSD, during both ontogeny and adulthood, may equip tarrkawarra for life-long fluctuations in local conditions and optimal mating strategies.

INTRODUCTION

There is a widespread tendency among animals for one sex to out-weigh the other (sexual size dimorphism, 'SSD') and in most invertebrate taxa female-biased sexual size dimorphism (FSSD) predominates. However, 45% of mammal species exhibit male-biased SSD (Lindenfors et al. 2007). Current theory asserts that male-male competition selects for larger male size in mammals while trade-offs between growth, lifetime fecundity and gestation and lactation costs are thought to constrain female size (Lindenfors et al. 2007). In some mammalian species with FSSD, the absence of direct male-male competition may relax selection on large male size, but this does not explain why female size is unconstrained (Isaac 2005). Excluding species with unique physiological constraints (e.g. flight in bats: Welbergen 2010), the same constraints on female size as seen in male-biased SSD should exist in FSSD mammals, otherwise selection on large female size must outweigh those costs (Blanckenhorn 2000).

The genome of the sexes is usually insufficiently divergent to account for SSD alone and only very few mammals show any detectable level of SSD at birth (Badyaev 2002). Instead, it is generally accepted that SSD results from divergent ontogenetic processes and that weight at weaning or sexual maturity is a good predictor of lifetime reproductive success (Lindström 1999). Although selection on size can also occur prior to sexual maturity (for example, via predation or viability), early growth typically also relates functionally to the benefits of different weights at first reproduction (Badyaev 2002). For example, in Primates (as an Order), sexual differentiation occurs relatively late in ontogeny or non-synchronistically with the other sex. Early, rapid and long

female growth spurts facilitate early pregnancies, whilst short and delayed male growth spurts prevent males being prematurely subjected to the risks of intra-sexual competition with older males (Badyaev 2002). The benefits of early sexual differentiation in growth are harder to interpret. For example, male pinniped pups, although not preferentially nursed over female pups, still grow larger due to a lower metabolic rate (Badyaev 2002). Trade-offs between breeding as early as possible and attaining the optimal size at which to breed determine the asymptotic weight of each sex.

The ontogeny of SSD (male or female biased) is inadequately described in many systems, arguably in none more so than FSSD mammals. These systems are, however, critical to our understanding of SSD and particularly to our understanding of the evolutionary precedents of SSD. Sexually differentiated size is often closely linked to the mating system of a species (e.g. larger males defend harems better than smaller males in polygynous systems), but mating optima fluctuate with ecological conditions that influence the number and spacing of available mates (Schradin et al. 2010; Schradin & Pillay, 2005). Quantifying rapid change and flexibility in SSD under variable ecological conditions is critical to our understanding of how the phenomenon evolves.

At a proximate level, mammalian sex-differences in size generally result from post-natal sex-differentiated growth trajectories. Pre-natal growth is very seldom sex-differentiated before birth but SSD is often present by weaning (Badyaev 2002). However differences in both growth rate and duration may continue long after weaning and sexual maturation (Székely 2007), often following non-linear trajectories, especially

in long-lived animals (Badyaev 2002; Fairbairn 2007). Despite this, evidence of sexual maturation is the usual standard for identifying 'adult' specimens (Fairbairn 2007). Depending on the age distribution of the population sampled the direction and degree of SSD is probably, at least occasionally, misrepresented based on these data (Smith & Jungers 1997). For instance, this could occur whenever one sex undergoes slow sustained growth up to and beyond sexual maturity, relative to the rapid growth of the other sex, which achieves asymptotic growth sooner (Fokidis et al. 2007; Garel et al. 2009; Jackson & van Aarde 2003; Welbergen 2010). Theoretically, sexual size parity may therefore occur in these species once both sexes reach asymptotic weights, despite SSD during youth (or vice versa).

Most data on the ontogeny of SSD are derived from species with well-established male-biased SSD. In these systems, sexual size differentiation is often supposed to occur, in part, because, relative to males, females trade increased size off against rapid growth and shorter periods to first reproduction (Garel et al. 2009; Jackson & van Aarde 2003). In turn, constraints on female birthing capacity are thought to prevent sexual-differentiation of birth weights (Lindenfors et al. 2007). However, the 'grow fast, breed early' strategy is intuitively much better suited to males (especially mammalian males) as they are free from selection on increased size to sustain egg production, gestation and lactation (Lindenfors et al. 2007). 'Grow fast, breed early' is the typical male pattern in invertebrate taxa, despite levels of male-male competition commensurate with many male-biased SSD mammals (Isaac 2005).

A reasonable number of mammals with FSSD have been reported (Ralls 1976), but few, if any, studies have tested at what point growth trajectories for the two sexes

diverge. It is therefore not yet possible to determine whether the above assumptions about female size selection and constraints vary in FSSD species, relative to each other and relative to non-FSSD species.

Two promising findings make tarrkawarra (*Notomys alexis* or spinifex hopping mouse) a potentially excellent system in which to investigate the ontogeny of FSSD: Firstly, there is evidence in captive tarrkawarra that the species is FSSD from shortly after weaning (15-25 days) until at least six months of age (Breed 1983; Dewsbury & Hodges 1987; Smith et al. 1972); Secondly inter-population variation in degree of FSSD has been reported from the field (Bauer & Breed 2008; Breed 1979, 1992). This allows within-species comparisons of the potential ecological or behavioural factors that contribute to FSSD.

The occurrence of FSSD in tarrkawarra requires further validation, however. Although young, captive tarrkawarra are FSSD, the species lives up to six years in captivity. Tarrkawarra males and females reach sexual maturity at about the same age (Breed 1979), but sex-specific growth trajectories after this may hypothetically result in sexual size parity in later adulthood. This scenario is supported by contradictory field data, showing FSSD in some populations and sexual size parity in others (Bauer & Breed 2008; Breed 1979, 1992). Trapping tarrkawarra in its arid zone habitat is largely unsuccessful except for brief periods several months after rain when population-densities increase as much as 40 to 100 fold (Dickman et al. 1999; Masters 1993; Predavec 1994). Not surprisingly, most wild tarrkawarra weight records date from these events. A combination of longevity, ‘bottle-neck’ population fluctuations and spot-

sampling of different field populations may produce an inconsistent degree of FSSD simply because age is seldom normally distributed (Predavec 1994).

Alternatively, the inconsistent degree of FSSD in wild-trapped tarrkawarra may be evidence for inter-population variation in FSSD. Specifically, it is only at high population-densities that wild-trapped tarrkawarra do not show FSSD. Females in low population-densities are 8-13% heavier than males (compare means listed in Bauer & Breed 2008; Breed 1979, 1992). Similar within-species, between-population correlations of density and degree of SSD have been reported for other mammals (Bonenfant et al. 2002; Haisova-Slabova et al. 2010; Hewison et al. 2002; Johannesen & Andreassen 2008; Rittschof 2010). These are usually attributed to either differences in forage availability and / or differences in the optimal mating system at different population-densities (Schradin et al. 2010; Schradin & Pillay 2005).

Forage availability is usually assumed to be lower at high population-densities because of increased competition (Isaac 2005), potentially reducing animal weights and altering the degree of SSD. However, there is evidence that tarrkawarra populations begin to decline prior to declines in food supply (Dickman et al. 2010). In accordance with this, sexual size parity in high-density populations is observed because, relative to low-density populations, mean male weights are greater (Bauer & Breed 2008; Breed 1979, 1992; but see also Predavec 1994). Fluctuating mean male weights in the field may therefore be related more directly to the variable social dynamics dependent on population-density itself, than to forage availability *per se*. For example, at low densities females are few and far between and small, nimble males that search and scramble for females may achieve greater fitness. In high population-densities,

defending access to a spatially clumped group of females becomes feasible provided a male can defend against other males – usually by having a size advantage. In tarrkawarra, recent field work has indicated the potential for flexible social organisation at different population densities (Dickman et al. 2010), or, at least inconsistent social organisation between populations (Bradley 2009). However, there is a well-established, profound absence of pre or post-copulatory competition in this species (Bauer & Breed 2006; Breed & Adams 1992; Happold 1976; Peirce & Breed 2001; Stanley 1971). Male defense polygyny is therefore unlikely to be a dominant feature of this species. Instead we infer varying levels of scramble competition, with strong selection on small male size at low-population densities and relaxed selection on large male size at high-densities. Scramble competition is consistent with the transient residency patterns indicated by low tarrkawarra recapture rates, high burrow desertion rates, the long-distances tarrkawarra are known to roam over and the dramatic variations in spatial distribution of their forage over intra-generational time (Bradley 2009; Dickman et al. 1995; Predavec 1994).

Here we consider sex-differentiation of tarrkawarra growth trajectories, from birth through to asymptote, in order to quantify a) how early FSSD becomes established, b) whether FSSD is maintained beyond adolescence into late adulthood in age-matched groups and c) if the degree of FSSD differs in tarrkawarra groups subjected to either high or low-density housing during the critical post-weaning to sexual maturation period. Based on previous captive studies (Breed 1983; Smith et al. 1972) we expected FSSD to be detectable by weaning although probably not significant until shortly after. Although early weights often scale to adulthood (Rödel et al. 2008), there are no firm data beyond six months of age confirming this to be the case in this

species. Based on other mammalian systems, we predicted the continuation of sex-differentiated growth rates beyond sexual maturation, with the degree of FSSD possibly being attenuated by 'catch-up' male growth rate 'spurts' or an extended duration of male growth to asymptote. Extrapolating from the available field data (Bauer & Breed 2008; Breed 1979, 1992; Predavec 1994), we also predicted the degree of tarrkawarra FSSD might reduce under high compared with low-density housing despite equal forage availability.

METHODS

Animals and Data Collection

Tarrkawarra are a semi-fossorial rodent species endemic to the central arid zone of Australia. The species has drawn comparison with a number of well-studied North American and African desert rodents with hopping gaits, but amongst which females are not larger than males (Randall 1994). Females produce four-pup litters (on average) a minimum of 32 days after mating (Breed 1989; Happold 1976; Smith et al. 1972). However, gestation may be prolonged up to 48 days due to delayed implantation (Breed 1979) although it is not clear why this occurs. Reproductive dormancy is typical of adult females (but not males) at the peak of boom and bust population cycles in the field (Bauer & Breed 2008; Breed 1992). However, clear evidence of any mechanism for this occurring in captive groups (e.g. reproductive suppression by dominant, older or related animals) is inconclusive (Bradley 2009). Female sexual maturation precedes that of males by no more than a few days (Breed 1979). This and other stages of development are listed in Table 1 and are not otherwise known to be remarkably sex-differentiated.

Weights of 89 neonates from 20 litters (three to six pups each, 42 female, 47 male) were analysed to assess pre-natal factors contributing to FSSD in tarrkawarra. To assess post-natal growth we collected 29 further weight observations (see below) for each of the 62 pups that survived to weaning age. All pups were born into a captive colony at Macquarie University, Sydney, Australia, 2004-2009 (Animal Ethics Protocol # AEC2005018). Pups represented the offspring of thirteen dams and nine sires, all less than 12 months of age at mating. Sires serviced one to three females and produced one to four litters each whilst dams were consistently paired with the same males and produced one to two litters of full siblings each. Matings were either witnessed and/or pairs separated such that paternity and gestation length were known. Gestating females were checked at least twice daily. As promptly following parturition as possible we collected the following data for each new litter: days gestation; number of pups ('litter size'); individual pup mass (g); total combined pup mass ('litter birth mass'); and likely sex ratio (male pup count/total pup count). Litter sex ratio was determined conclusively later, once pup genitalia were more pronounced. Pre-weaning mortality and/or infanticide is common in this species (Breed 1979; Smith et al. 1972) and several litter sizes in this sample reduced in the first five days (from a mean of 4.45 to 4.10 pups), often also altering the litter sex ratio (from a mean of 0.54 to 0.56, male pup count/litter pup count). After day 5, litter size was consistent through until to weaning in all but one case. To control for different pre and post-natal litter composition effects, we used reduced litter size, litter mass and sex ratio figures in all but the neonate analyses. In the only litter where pup mortality occurred later (day 14), figures were not adjusted.

Individual tail markings identified pups until their pelts developed enough for unique patches to be clipped and until genitalia were sufficiently developed for them to

be conclusively sexed. Individual weights were collected at regular intervals for each animal up to 1000 days of age or until death, whichever occurred first (Table 1). Six females in the sample produced offspring during the trial and their weights whilst gestating and nursing were excluded from the dataset. All weights were accurate to within 0.1g (Ohaus scale, model JR300).

Table 1. Schedule of ages at which animals were weighed by developmental stage

Developmental stage	Age / age range (days)	Days between weigh-ins	Observations per stage
Parturition*	Day '0'/birth	-	1
c. One week	5 – 10	5	2
Weaning*	15 – 25†	5	3
c. Six weeks	30 – 50	10	3
Sexual Maturation*	60 – 80	10	3
c. Four months	100 – 160	20	5
Six Months*	180	-	1
c. Nine months	200 – 300	50	3
Twelve months*	350	-	1
c. Fifteen months	400 – 450	50	2
Eighteen months*	500 – 600	100	2
Twenty-four months*	700 – 800	100	2
Full Adulthood/Final Weight*	900 – 1000	100	2

**Stages included in data analyses. Observations were averaged where >1. Note that observations per stage vary and in some cases (where observations = 1) represent a 'point in time' measurements, in others an average across a period of time.*

†Weaning begins at 15 days when first solid foods are investigated and mouthed. It may be completed at 15 days if dam dies (pers obs) but typically takes a further 7-10 days.

Animal Housing

Natal housing was in small groups (one to two adults plus young), in solid-bottom polycarbonate cages (50 × 40 × 40 cm, L × W × D) with paper pellet substrate, hides, nest boxes and tissue bedding. Immediately post weaning, pups were re-housed into either 'low' or 'high' density housing conditions. Low-density enclosures held two to five individuals, high-density enclosures held nine to 11 individuals. Enclosures were 11m^2 polycarbonate walled indoor pens on concrete floors with sand substrate, furnished with branches and hollow log hides (Fig. 4). Low versus high-density weaning weights were not significantly different within-sex (LMM, estimated marginal means: females, $F_{1,220.4} = 1.48$, $P = 0.23$; LMM2: males, $F_{1,221.2} = 0.004$, $P = 0.95$). The housing density manipulation was maintained until the pups were six months of age, at which point random density housing applied to all animals. Temperature and lighting were controlled throughout to 20-23°C and 14:10 h light:dark cycle. Access to water and food (millet seed mix and fresh carrot cubes) was *ad libitum.* Cages and enclosures were cleaned at least fortnightly.

Statistical Methods

To account for non-independence of litters, a random term ('litter cluster') was included in all statistical models unless otherwise noted. Litters in the eight clusters may have been born to different dams but were all sired by the same male (see above). This form of clustering was found to best capture the lack of independence due to litters/parents.

Neonatal Weights & Gestation Factors

To analyse sex differences in individual neonate mass, we employed a linear mixed model (LMM 1a), with pup sex, litter gestation length and the three litter composition

variables (litter size, mass and sex ratio) as fixed factors. We ran the LMM (1a) analysis twice, once including and once excluding (LMM 1b) pups that did not survive to weaning.

Next we ran three bivariate correlation analyses (Spearman rank) to investigate the association between gestation length (32-48 days) and each of the three litter composition variables. To avoid pseudo replication, we used litter means in these analyses and excluded the litter cluster variable used in the other analyses (see above). Four litters were excluded because their mothers were nursing a previous litter during their gestation.

Birth and Weaning Weights as Predictors of Adult Weight

To estimate the relative contributions of pre-natal and pre-weaning development on adult weights, we ran regression analyses for each sex firstly on birth weights and then on weaning weights with weights during sexual maturity and full adulthood.

Density and Degree of Sexual Size Dimorphism

We created a second linear mixed model (LMM2) to test mean weight differences for the four developmental stages before (start of weaning), during (at sexual maturity and at six months) and after (twelve months) the density manipulation. Sex, developmental stage and density condition (low or high) were included as fixed factors and we also tested the two and three-way interactions of these variables. To control for early growth factors we also included litter mass at birth and gestation days as fixed factors, centered at their means. To account for repeated measures, we used a multilevel model with a

random intercept for each animal at level one and a random term for 'litter cluster', at level two.

Growth Curves from Birth – 1000 Days

To establish how growth changed over time for each sex, and to account for uneven weight-collection intervals, we calculated daily growth rates (DGRs) for each developmental stage. To do this we divided each animal's weight change during each developmental stage (e.g. weight at sexual maturity minus weight at weaning) by the number of days in that stage (e.g. weight change from weaning to sexual maturity/25 days). We used a third linear mixed model (LMM3) to identify changes in mean female and male DGRs over time and to establish at which stages DGRs differed significantly between the sexes. This model was essentially identical to LMM2 (see above) except that it analysed DGR, rather than weight, and was used on a dataset including all developmental stages. Tests of estimated marginal means indicated at which stages significant differences in DGR occurred.

All analyses were conducted using SPSS, version 16.0. Normality of data distribution was established via kurtosis tests for skewness and, where necessary, data were centered to the mean. A significance level of $\alpha = 0.05$ was used for all tests and all values reported are means ± standard error unless otherwise noted.

RESULTS

Neonatal Weights & Gestation Factors

Female pups were significantly heavier than males at birth (LMM1a: $F_{1,89} = 24.15$, $P < 0.0001$) with an estimated mean weight of 2.46g, 13.8% heavier than males. Removing data from pre-weaning mortalities made little difference (LMM1b: $F_{1,62} = 24.84$, $P < 0.0001$), except to increase the estimated mean weights, especially of females (mean = 2.66g, males 17.2% lighter). Effects of the model's other fixed factors (litter gestation length and the three litter composition variables) were analysed via litter-level regression analyses (Fig. 1) to avoid pseudo-replication. These showed that gestation length did not vary with pup count ($R^2 = 0.03$, $F_{1,14} = 0.47$, $P = 0.51$) but longer

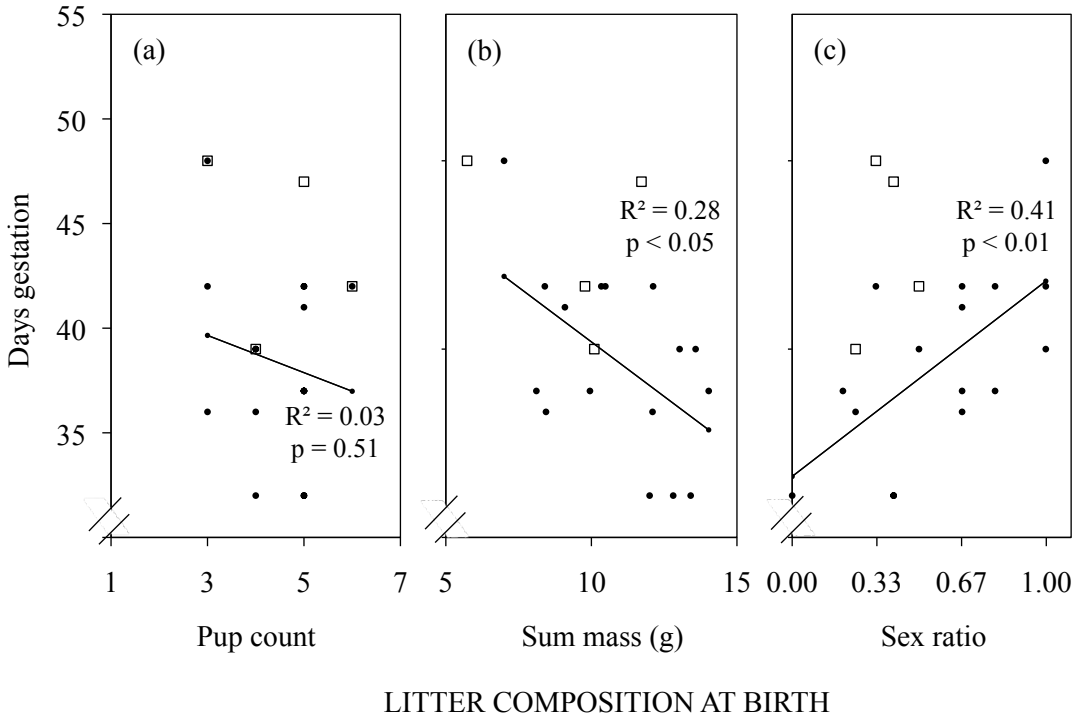

Figure 1. Litter gestation length for 16 litters in relation to litter composition factors: (a) number of pups per litter (conventional litter size); (b) total litter mass at birth in grams; (c) litter sex ratio (proportion of male pups). Four litters whose dams were simultaneously nursing a previous litter during gestation were excluded from analyses but are shown with open squares.

gestation periods did result in significantly lighter litters at birth ($R^2 = 0.28$, $F_{1,14} = 0.28$, $P < 0.05$). Sex ratio also increased significantly with gestation length ($R^2 = 0.41$, $F_{1,14} = 0.41$, $P < 0.01$), indicating that lighter litters had proportionally more male than female pups. The interaction between litter birth mass and sex ratio was marginally non-significant ($R^2 = 0.24$, $F_{1,14} = 4.39$, $P = 0.055$).

Birth and Weaning Weights as Predictors of Adult Weight

Birth weight of males predicted 42.1% of weight variance at sexual maturity ($R^2 = 0.42$, $F_{1,31} = 22.5$, $P < 0.001$) although it only predicted 18.9% of variance in full adulthood ($R^2 = 0.19$, $F_{1,25} = 5.84$, $P < 0.05$). Female birth weight was in no way predictive of weight at sexual maturity or in full adulthood ($R^2 = 0.05$, $F_{1,25} = 1.42$, $P = 0.25$; $R^2 = 0.05$, $F_{1,22} = 1.21$, $P = 0.28$ respectively). However, female weaning weight was related to adult weight: weaning weights predicted 49.9% of weight variance at sexual maturity ($R^2 = 0.50$, $F_{1,25} = 24.86$, $P < 0.0001$) and 64.4% of weight variance in full adulthood ($R^2 = 0.64$. $F_{1,22} = 39.74$, $P < 0.0001$).

Density and Degree of Sexual Size Dimorphism

Sexual size dimorphism (as measured by sex differences in weight) was significantly affected by both developmental stage and housing density (LMM2: $F_{1,221.2} = 5.28$, $P < 0.05$; $F_{3,216.4} = 5.39$, $P < 0.001$, respectively). Specifically, in low-density housing, differences in estimated marginal means (Table 2) indicated that FSSD increased over the course of the manipulation and was maintained after the housing manipulation ended at six months (Fig. 2a). However, FSSD under high-density at the start of the manipulation (weaning), disappeared by sexual maturity. Sexual size parity seemed to be the result of high-density females gaining significantly less weight than low-density

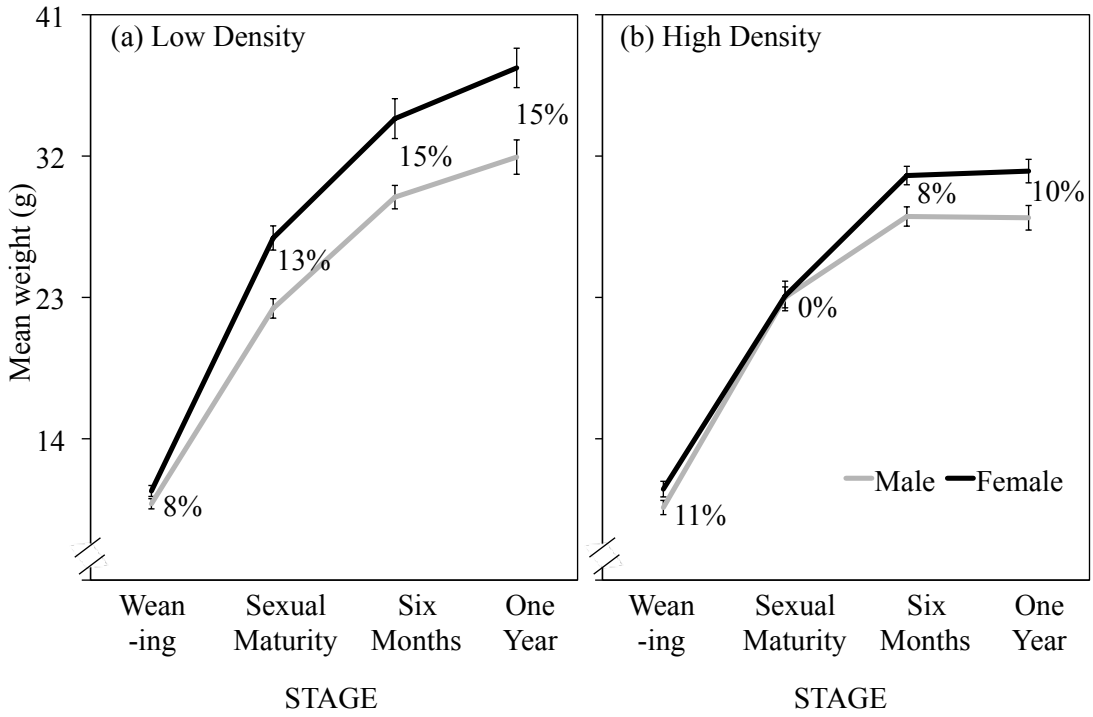

Figure 2. Female (black lines) versus male (grey lines) weights throughout the housing density manipulation. Lines track mean weights (bars indicate standard errors) from the start of the housing density manipulation (weaning), during the manipulation (sexual maturity and at six months of age), to six months after its termination (one year). Percentages indicate degree of FSSD (female minus male weights) at each stage (rounded). Two-way interactions of sex, developmental stage and housing density were all significant effects on animal weight (see text for details) although the combined effect of sex, developmental stage and housing density was marginally non-significant (LMM2: $F_{3, 216.8} = 2.50$, $P = 0.06$).

females (Table 2, Fig. 2b). Under high-density, FSSD re-emerged by the end of the manipulation, but high-density females continued to be significantly lighter than low-density females (Table 2). The only significant difference between high and low-density male weights occurred at twelve months of age, six months after the housing manipulation ended (Table 2) with low-density males being larger than high-density males.

Table 2. Within and between sex weight differences during the housing density manipulation.

Group	Weight Differences at Developmental Stage‡		
	Sexual Maturity	Six Months	Twelve Months
Low-density	4.02g	4.55g	5.50g
Female-Male	$F_{1,219.1}$ = 20.28***	$F_{1,220.0}$ = 21.82***	$F_{1,220.5}$ = 31.54***
High-density	0.912g	2.592g	2.904g
Female-Male	$F_{1,218.2}$ = *0.92*	$F_{1,217.4}$ = *8.44***	$F_{1,216.9}$ = 10.10**
Female	2.51g	2.62g	5.75g
Low-High density	$F_{1,220.2}$ = *6.09**	$F_{1,219.7}$ = *6.90***	$F_{1,218.8}$ = 33.30***
Male	-0.060g	0.66g	3.17g
Low-High density	$F_{1,221.1}$ = 0.48	$F_{1,222.7}$ = 0.55	$F_{1,223.0}$ = 11.95**

*p-values indicated as follows: * < 0.05, ** < 0.01, *** < 0.0001*

‡ Estimated marginal means.

Growth Curves from Birth – 1000 Days

Changes in daily growth rate (DGR) between developmental stages were significantly different for males and females (LMM3, sex by stage: $F_{6,374.3}$ = 3.50, P < 0.005). From birth through to sexual maturity mean female and male DGRs were 0.26g and 0.23g per day, respectively, and tests of estimated marginal means (EMMs) indicated these differences were significant ($F_{1,379.6}$ = 4.41, P < 0.05). From sexual-maturation to twelve months of age, mean female and male DGRs both slowed significantly (to means

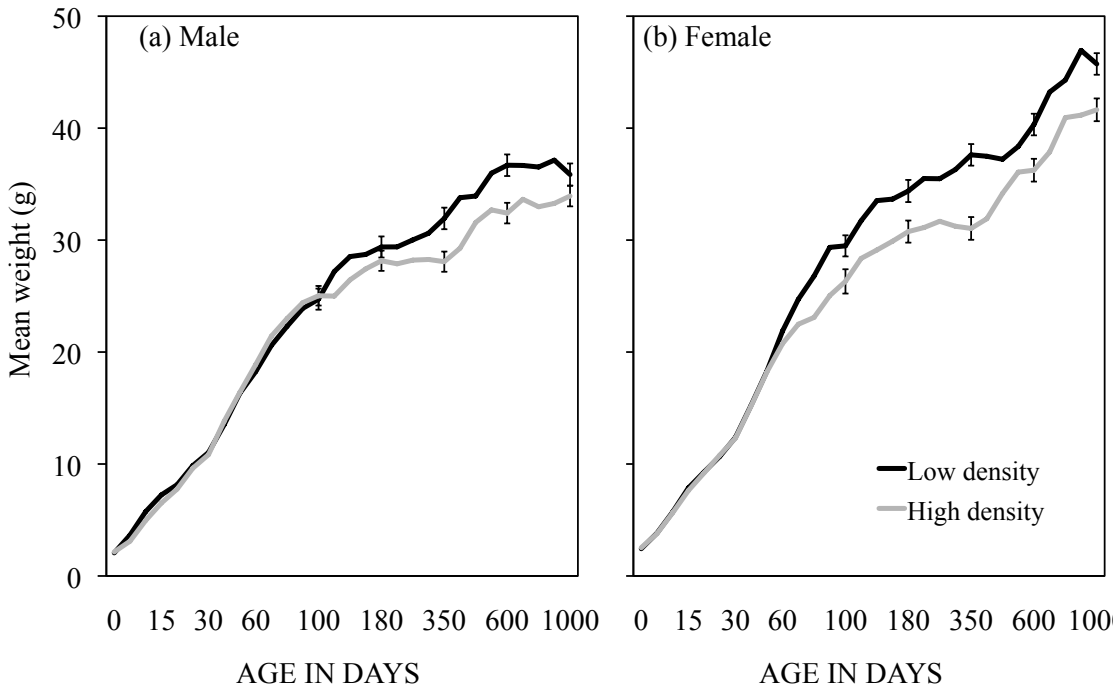

Figure 3. Male (a) and female (b) growth from birth, through weaning (21-30 days), to sexual maturity (60-80 days), and through to 1000 days of age. Black lines indicate mean weights of animals housed in low-density conditions, grey lines mean weights of animals housed in high-density conditions from weaning to six months of age. Bars indicate standard errors of the means.

of 0.039 and 0.036g respectively) and were approximately equal from six to 12 months of age (EMMs: $F_{1,379.9} = 0.18$ $P = 0.67$). However, whilst mean female DGR did not reduce significantly again, mean male DGR did. From 18 to 24 months further significant drops in mean male DGR (zero from 24 months on) reinstated the significant sex-difference in DGR (EMMs: $F_{1,380.3} = 7.82$ $P < 0.01$) through until the end of the study (Fig. 3).

Density alone had a barely significant effect on DGR over time (LMM3, density by stage: $F_{6,376.2} = 2.07$, $P = 0.06$) but it was important in combination with sex (LMM3, sex by density by stage: $F_{6,374.9} = 3.83$, $P < 0.002$). Tests of estimated marginal means (EMMs) indicated density condition was a marginally non-significant effect on mean male DGR only during the first part of the housing manipulation (EMMs weaning-sexual maturity: $F_{1,381.2} = 3.74$ $P = 0.054$). Mean high-density DGR was also less at this time for females, compared with low-density counterparts ($F_{1,380.8} = 11.70$, $P < 0.002$). However, well after the housing manipulation high-density females experienced a significant, albeit temporary (from 12-18 months), increase in growth relative to low-density females ($F_{1,378.7} = 4.57$, $P < 0.05$).

DISCUSSION

FSSD was observed in this sample at birth, at every developmental stage, and under nearly all conditions, thereafter. Females were born significantly heavier than males but birth weights predicted only adult male and not adult female weights. As well as being heavier at birth, female daily growth rate to weaning was also greater than males'. Duration of growth also varied between the sexes with females continuing to increase in

weight throughout the study (nearly three years) whilst males approached asymptote at approximately 18 months of age. We observed no ‘catch-up' male growth to reduce FSSD as the population aged. The only factor that appeared to reduce the degree of FSSD in this study was post-weaning housing density. However this was the result of retarded growth, predominantly in females, under high-density conditions, rather than increased male growth to parity with females, as predicted based on field data.

Sex-differentiated pre-natal development resulting in SSD from birth is considered unusual (Badyaev 2002), although the relationship between neonate weight and adult SSD is seldom actually studied (Smith & Leigh 1998). Female tarrkawarra in this study were approximately 14-17% heavier than males at birth and nearly 13% heavier at weaning.

Sex-differentiated pup birth weights may be related to the variable gestation periods found in this species, as longer gestations resulted in significantly lighter natal litter mass and proportionately more male than female pups on average. Although beyond the scope of this paper, these findings suggest that neonatal FSSD in tarrkawarra may be related in some way to litter sex-bias. Sex-biased litters should be adaptive in polytocous animals if local intrauterine conditions promote the growth of one sex of fetuses at the expense of the other (Grant & Chamley 2010). Relationships between offspring fitness and litter sex ratios in a number of species support the possibility of sex-biased maternal investment, in some cases via sex ratio manipulation (Koskela et al. 2009; Clark & Galef 1995; Clark et al. 1992). Polytocous animals in general produce more sex-skewed litters than predicted by chance (James 2009) and a number of mechanisms for sex ratio manipulation have been identified (Navara &

Nelson 2009). In particular, studies of other mammals implicate sex-differentiated delayed implantation may be related to circulating glucose, testosterone or corticosteroid levels at the time of conception (Navara & Nelson 2009). Delayed implantation is known to be associated with lengthy gestation periods in tarrkawarra (Breed 1979). Our finding that gestation length and litter sex ratio are also related therefore suggests sex-differences in delayed implantation in this species. Given that male pups are smaller than female pups at birth, any mechanism producing sex-differentiated delayed implantation might also affect litter sum mass. Further research on the precedents of delayed implantation in this tarrkawarra would be a highly worthwhile contribution to the literature on this phenomenon in mammals.

Further research is also merited on the effects of litter sex ratio on growth and reproductive fitness in tarrkawarra. Sexual behaviour, sex ratio of subsequent offspring and reproductive fitness are all know to vary according to the litter sex ratio gerbils experience during gestation (Clark et al. 1992; Clark & Galef 1995a, b). These effects are attributed to different intrauterine exposure to circulating hormone levels according to sex and number of adjacent fetuses. Further research is therefore also warranted on the effects of litter sex ratio on the subsequent life history traits of pups and could harness the ease with which tarrkawarra cross-foster pups.

Weight in early development determines maximum possible growth rates, often scales to adult weights and, in turn, affects fitness (Lindström 1999; Rödel et al. 2008). Scaling of weight attained prior to parental independence also seems to be the case for tarrkawarra. For males in this study, birth weights were particularly important. This may reflect the fact that tarrkawarra dams have only four teats and pups practice tenacious nipple attachment (Breed & Ford 2007). In the jostle to obtain and retain prime nursing

positions, sibling competition probably favours heavier pups, especially in larger litters (Bautista et al. 2009). Further adding to this post-natal handicap on growth in smaller tarrkawarra offspring, observations from early adulthood indicate that male metabolic requirements exceed that of females, with males consuming significantly higher proportions of their mean body weight despite having lower daily growth rates (Ngahere unpublished data). Such impediments to growth may explain the diminishing predictive power of post-natal male weights on adult weight by contrast with improved post-natal weight scaling as female pups approached adolescence.

In some species sex-biased parental investment compensates the smaller sex for early growth handicaps via differential intrauterine resourcing or selective nestling provisioning (Badyaev 2002; Metcalfe & Monaghan 2001). No evidence of such mechanisms were found for tarrkawarra in this study, and combined with the absence of any 'catch-up' growth in adulthood, may indicate relaxed selection on male size. Conversely, early female growth rates and duration recorded here exceeded those of males, further enhancing the FSSD present at birth and suggesting strong, positive selection on female size. However, it remains to be shown whether adult size and fitness are in fact correlated in either sex.

Male growth in particular is known to be sensitive to fluctuating levels of sexual and viability selection in a number of species (Bonenfant et al. 2002; Hewison et al. 2002; Johannesen & Andreassen 2008; Leberg & Smith 1993; Oksanen et al. 2007; Rittschof 2010) and it seems a plausible explanation for the fluctuating degree of FSSD observed in free-living tarrkawarra. Because of frequent low population densities in the field, most tarrkawarra will have to undertake long-distance mate-searching at some

point in their lives in order to reproduce. Male scramble competition tends to select for locomotor and metabolic efficiency, rather than increased size (Cox 2010), and might explain why male tarrkawarra are smaller at low compared with high population densities. However, when female spatial distribution contracts (because forage is plentiful), male defense of multiple females may become a more viable mating strategy (Rittschof 2010). Indeed, the field data on FSSD in tarrkawarra appear to support scramble competition with a degree of social flexibility (Dickman et al. 2010; Dickman et al. 1995).

In this study, we confirmed flexibility in FSSD due to population density. Specifically FSSD was pronounced in the low-density housing and near sexual size parity characterised the high-density housing, as predicted by the field data. However, comparisons between the housing conditions did not reflect field observations of FSSD at different population densities. Sex differences in growth from weaning to six months produced depressed mean weights for both sexes in high compared with low-density housing. By contrast, at high field population densities, data collectively indicate comparable female and elevated male weights compared with low population densities (Bauer & Breed 2008; Breed 1979, 1992). Whilst standardising breeding conditions, forage availability and seasonal indicators such as photoperiod and temperature, our dichotomous captive housing conditions were obviously a tenuous simulation of field population variation. There are clear differences between crowding in captivity and high population density in the field and ecological boom and bust effects on population density and differential growth in the sexes are no doubt much more complex in the field. For example, at times of low-density population, corresponding drops in predator numbers, may increase tarrkawarra survival rates in the field and, in turn, increase the

mean population age (Yoccoz & Mesnager 1998). However, it is interesting to note that, in this species, female as well as male growth may be sensitive to local conditions and, specifically, population density.

At nearly 20% larger, tarrkawarra female size superiority can be dramatic by vertebrate standards. The SSD frequency distribution for mammalian and bird species is leptokurtic, with fewer such extreme cases in the 'tail ends' than predicted under a normal distribution (Ralls 1976; Székely 2007). Furthermore, female size bias exists from birth, and weight gained in the nursing period is especially critical to subsequent female growth. Further research is now required to determine the specific pre- and post-natal mechanisms involved in sexual size differentiation of tarrkawarra. Meantime, these findings collectively suggest greater fitness costs of poor growth for females than for males. Whilst not yet recorded in any FSSD species, in many other animals, the largest specimens of the larger sex may acquire more or better quality mates than smaller counterparts. Alternatively, increased maternal mass is often related to increased fecundity and offspring viability (Blanckenhorn 2005; Fokidis & Risch 2008; Fokidis et al. 2007; Wheatley et al. 2006). Whatever the selective agent, the final degree of FSSD in tarrkawarra can be not only dramatic but also dramatically variable in this species, depending at least in part on population density. These characteristics make tarrkawarra an excellent model system for further research on the conditions under which FSSD emerges in mammals. More broadly, the flexible level of FSSD in tarrkawarra may inform our understanding of evolutionary responses to fluctuating optimal mating strategies.

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Figure 4. Typical example of the 11m^2 floor pens used to house tarrkawarra in either high (nine to 11 animals) or low-density (two to five animals) conditions

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Chapter 3

Litter sex-ratio effects on female but not male growth in a small mammal with female-biased sexual size dimorphism

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ABSTRACT

When the cost of reproduction is considered, most theory surrounding sex-differentiated maternal investment does not adequately provide for mammals with female-biased sexual size dimorphism (FSSD). Specifically when females are the larger sex, male-biased maternal investment during optimal conditions is still predicted because the requirements of mammalian parental care constrain the maximum potential reproductive success of females more than males. However, to date, this has been tested in only one FSSD mammal that I am aware of (*Myodes glareolus*, Koskela et.al. 2009). I therefore analysed growth to sexual maturation in the FSSD tarrkawarra (*Notomys alexis* or spinifex hopping mouse) and found evidence of female-biased maternal investment in offspring. Specifically females from female-biased litter sex-ratios were heavier at sexual maturation than females from other litter sex ratios. By contrast, litter sex-ratio made no difference to the growth of male offspring. In support of female-biased maternal investment increasing fitness, I also found a positive correlation between female weight at sexual maturation and mean pups per litter subsequently produced. More data on FSSD mammals are required before sex allocation can be adequately explained by theory.

INTRODUCTION

Sexual size dimorphism (SSD) has future fitness consequences for offspring but also for their mothers. This should result in selection on maternal effects that are sensitive to environmental conditions that produce sex-differentiated growth and which contribute positively to the ontogeny of dimorphism (Badyaev, 2002). Sex-specific investment is amongst these maternal effects and is predicted when the fitness of one sex is likely to outstrip that of the other sex, given the prevailing conditions (Trivers & Willard 1973). One of the clearest and most common mammalian examples of this occurs in polygynous and male-biased sexually size dimorphic species. In these cases high quality sons are the most costly offspring to produce because their fitness depends on attaining maximal size during the period of parental investment (McDonald et al. 2005). Large sons are the best equipped for male-male competition and outstrip the reproductive success of lower quality, smaller sons and exceed the 'stable but low' reproductive output of daughters (Cameron 2004; Fairbairn 2007; Trivers & Willard 1973).

In response to sex differences in fitness potential observed in male-biased SSD systems, if conditions are optimal for the rearing of sons, dams tend to invest in the larger-sized sex. That is, they produce small male-biased litters under good conditions, but invest in larger litters of female offspring under poor conditions. This probably reflects the less variable reproductive success that typifies female over male offspring, even when reared under poor conditions (Krackow & Hoeck 1989; Stoehr & Kokko 2006; Zuk 2009). Sex-ratio bias may be achieved by sex ratio adjustment, for instance in animals where circulating glucose, testosterone or corticosteroids at the time of

conception may affect fetus implantation and viability in one sex more than the other (Navara & Nelson 2009). Alternatively the size and the sex ratio of the litter may produce post-natal maternal investment biases (Clark et al. 1990; Koskela et al. 2009). Sex-differences in sibling competition may further contribute to differences in the growth of offspring depending on the litter composition (Uller 2006).

The relative size of the sexes determines many of the predictions made with regard to sex-biased maternal investment. Should the direction of SSD be female rather than male-biased it consequently follows that, all things being equal, sex-ratio biases should also be reversed in favour of females when conditions allow. The idea that sex ratio adjustment and sex-biased maternal investment are driven by the relative size and reproductive success of the two sexes rests on the validation of this hypothesis (Robert & Schwanz 2010). However, sex ratios observed even in non-SSD and weakly male-biased SSD species indicate that sex-size differences alone cannot predict maternal investment strategies (Cameron 2004; Hewison et al. 2002; Schwanz 2008). Additionally these species are not necessarily differentiated from strongly male-biased SSD systems by the absence of polygynous mating systems (Bro-Jørgensen 2007; Dunham 2008; Dunham & Rudolf 2009; Vanpé et al. 2008). Female-biased sexually size dimorphic (FSSD) mammals are even more challenging to the idea that the Trivers-Willard hypothesis can be extended beyond its original 'narrow sense' interpretation. Mating systems are equally variable in FSSD species (Dammhahn & Kappeler 2005; Engh et al. 2002; Ralls 1976; Roberts & Dunbar 2000; Sachser et al. 1999; Schülke 2005; Schulte-Hostedde et al. 2004) and the high female costs of mammalian reproduction (gestation and lactation) suppress many aspects of sex-role reversal, where paternal exceeds maternal investment (Lindenfors et al. 2007). Most theory implicitly

requires that, for mothers to invest more heavily in female offspring during gestation or lactation, there must also be sex-role reversal leading high-quality female reproductive success to outstrip that of males and poor-quality females (Eens & Pinxten 2000).

Mammalian FSSD does occur in a range of taxa (Ralls 1976; Chapter 1). In tarrkawarra (*Notomys alexis* or spinifex hopping mouse), where females are up to 20% heavier than males (Bauer & Breed 2008; Breed 1979, 1983, 1992), there are also potentially elements of sex-role reversal, which is unusual for a mammal. Vigorous inter-sexual aggression occurs between females rather than males, possibly driven by limitations of male reproductive ability (Suttle et al. 1988). Specifically, males have exceptionally low testis:body mass (Breed 1982, 1986, 1997; Peirce & Breed 1989) demonstrate nest-helping behaviour (at least in captivity; pers obs, pers comm. W. Breed), suggesting low sperm competition and relatively high paternal investment. Additionally body mass at the termination of parental investment is much more predictive of female than male adult mass (Chapter 2) suggesting that female-biased maternal investment may indeed occur in this species.

As a preliminary investigation into whether there is likely to be sex biased maternal investment after birth in tarrkawarra I firstly, considered evidence that mothers invest differentially into litters based on litter size and litter sex ratio. I hypothesised that the typical trend of sex-biased maternal investment in male-biased SSD species (favoring increased allocation to male offspring) would be at least partially reversed in the FSSD tarrkawarra. Secondly, to validate any sex-ratio effects I also tested whether the adult size of females and males is related to their reproductive output, i.e., if any bias in maternal investment up to weaning is likely to have fitness consequences.

METHODS

Animals and Data Collection

The semi-fossorial tarrkawarra are endemic to the central arid zone of Australia where boom and bust population conditions result in highly fluctuating population densities. They bear similarities to hopping rodents found in the deserts of North America and Africa, such as kangaroo rats, gerbils and jerboas (Dickman et al. 1999; Randall 1994). However, unlike these other species, the females of a population are often larger than the males. Females produce an average of four pups per litter, as early as 90-110 days of age, after a 32-48 day gestation period (Breed 1989; Happold 1976; Smith et al. 1972). The high variability in gestation period is attributed to delayed implantation (Breed 1979). Pups are self-sufficient by 20-30 days of age and may live up to six years in captivity (pers obs.).

Data were collected from captive animals held in controlled conditions at Macquarie University, Sydney from 2004-2009 (Animal Ethics Protocol # AEC2005018). All animals were housed in solid-bottom polycarbonate cages (50 × 40 × 40 cm, L × W × D) with paper pellet substrate, hides, nest boxes and tissue bedding. Pups were removed from natal groups (one to two adults plus offspring) shortly after weaning (30 days) and re-housed with same-sex litter-mates and/or unrelated opposite-sex individuals. Temperature and lighting were controlled throughout to 20-23°C and a 14:10 h light:dark cycle. Access to water and food (millet seed mix and fresh carrot cubes) was *ad libitum* and cages were cleaned at least fortnightly.

All animals were individually identified from birth by tail markings and, later, by fur clippings. Growth from birth to sexual maturity was measured every five days from 0 - 30 days of age and every 10 days from 40 - 80 days of age. Weights were accurate to 0.1g.

Offspring

A total of 62 tarrkawarra pups (27 female, 35 male), of known paternity, from 16 litters of three to five pups were used in this study. I used litter sex-ratio and total litter mass at birth (day 0) to group pups for analysis. Conventional sex-ratio (male/total pups) and litter pup count were partially confounded in this sample, as there were no five-pup litters with a sex ratio less than 0.6. That is, all of the largest litters also had a male-biased sex ratio. To be able to address the main questions, I therefore deemed pups with more same than opposite sex siblings to be in 'own sex-bias' and otherwise in 'other sex-bias'. If total litter mass (in grams) at birth (hereafter 'litter birth-mass' or 'litter mass') was below the median, I deemed pups as being from 'small litter-mass' (three to four pups), and otherwise from 'large litter-mass' (four to five pups). Preliminary analyses suggested that total litter mass but not litter size (pup count) was meaningfully related to other variables. Pup count was constant from day 0 to day 15 (the onset of weaning, when solid foods are first investigated and mouthed) in all but two litters. In the first of these, pup-count was reduced by one female at day 2 (cross fostered out due to maternal aggression) and, in the second, by two female pups at day 13 (disappeared). Only pups remaining at day 15 were included in the analysis but, to best reflect pre-weaning milk competition, I included the disappeared pups in litter sex bias and litter mass calculations.

Breeders

The breeders included 11 dams and eight sires mated to produce the above offspring. Average age at reproduction was 6.71 (± 3.24 SD) and 7.44 (± 2.83 SD) months respectively. Dams produced one or two litters each with the same sire. Sires produced up to four litters each with one or two females. For each breeder I recorded reproductive output in three categories: mean pup weights at birth, weaning and sexual maturity, mean number of pups per litter and mean litter sex-ratio (male/total pups).

Statistical Methods - Offspring

To explore the relationship between litter composition and weight gain to sexual maturity, I employed a linear mixed procedure and carried out a full factorial analysis with sex (2 levels), litter sex-bias (2 levels), litter birth-mass (2 levels) and days of age (12 measures) as fixed effects. I used a multilevel repeated-measures model including a random intercept for each animal at level one. In addition I included a random term for 'litter cluster', at level two. Litter cluster grouped offspring by sire to account for non-independence of litters and adequately reflected the main source of variation at the third level of the model.

Statistical Methods - Breeders

To determine the relationship between early development and subsequent reproductive fitness, I ran regression analyses on the weaning and sexual maturity weights of dams and sires, with the mean number of pups in litters they subsequently produced. Weights on days 15, 20 and 25 and on days 60, 70 and 80 respectively, were averaged to estimate weaning and sexual maturity weights. I also ran a regression analysis on mean number of pups with the mean sex-ratio of those litters and another regression analysis

on mean dam weights with mean sex-ratio of the litters they produced. Data were transformed to correct for skew where necessary and all analyses were conducted using SPSS, version 16.0.

RESULTS

Offspring Growth Curve Analysis

The four-way interaction of sex, litter birth-mass, litter sex-bias and days of age was significant ($F_{11,674.0} = 1.94$, $P < 0.05$), indicating that the litter sex-bias pups are born into affects female more than male weight gain to sexual maturity.

Because of the fallibility of four-way interactions, I isolated specific effects incorporated in the four-way interaction by examining pair-wise comparisons of estimated marginal means. I particularly focused on within-sex weight differences, and report indicative results with reference to the three critical developmental stages – birth, weaning and sexual maturity.

Regardless of litter birth-mass, female pups in female-biased litters were significantly heavier than those in non-female-biased litters from shortly after weaning onwards (day 40: $F_{1,79.2} = 4.01$, $P < 0.05$; Figs. 1(a) and 1(b)). The difference between females in female-biased litters of smaller versus larger birth-mass was significant from sexual maturity (day 60) onwards, with those from litters of smaller birth-mass being significantly heavier ($F_{1,123.7} = 18.27$, $P < 0.0001$; Figs. 1(a) and 1(b)). The combined effect of litter sex-bias and litter birth-mass on female pups was such that a female, reared in a small litter with fewer brothers than sisters, was 9.5 - 22.3% (2.4 – 5.1gm)

heavier at 80 days of age than females reared in other litter compositions. By contrast neither litter sex-bias nor litter birth-mass, made any significant or enduring difference to the growth of male pups (Figs. 1(c) and 1(d)).

Breeder Reproductive Success Analysis

Analysis of offspring data indicated that pups are heavier in litters of smaller birth-mass ($F_{2,59.9} = 3.71$, $P < 0.05$). Additionally, I found that whilst heavier dams had lighter pups ($R^2 = 0.05$, $F_{1,60} = 4.40$, $P < 0.05$, all weights at sexual maturity), they produced more of them. Dam weights at both weaning and sexual maturity correlated positively and significantly with the mean number of pups per litter they subsequently produced ($F_{1,9} = 6.03$, $P < 0.05$). There was no commensurate reproductive advantage for sires attaining heavier weights by sexual maturity ($F_{1,6} = 0.49$, $P = 0.51$; Fig. 2(a)). However sire weights at sexual maturity predicted one aspect of reproductive output. The heavier a male, the more sons he produced relative to daughters. ($F_{1,6} = 16.16$, $P < 0.01$; Fig. 2(b)). This was not the case for dams.

Stepwise backward reductions of non-significant terms were also preformed but did not explain the data any further.

DISCUSSION

I found that female pups born into female-biased litters achieve heavier weights at sexual maturation than those in other litters whilst litter sex-bias does not predict male weights at sexual maturation or during growth. Females born into small, female-biased

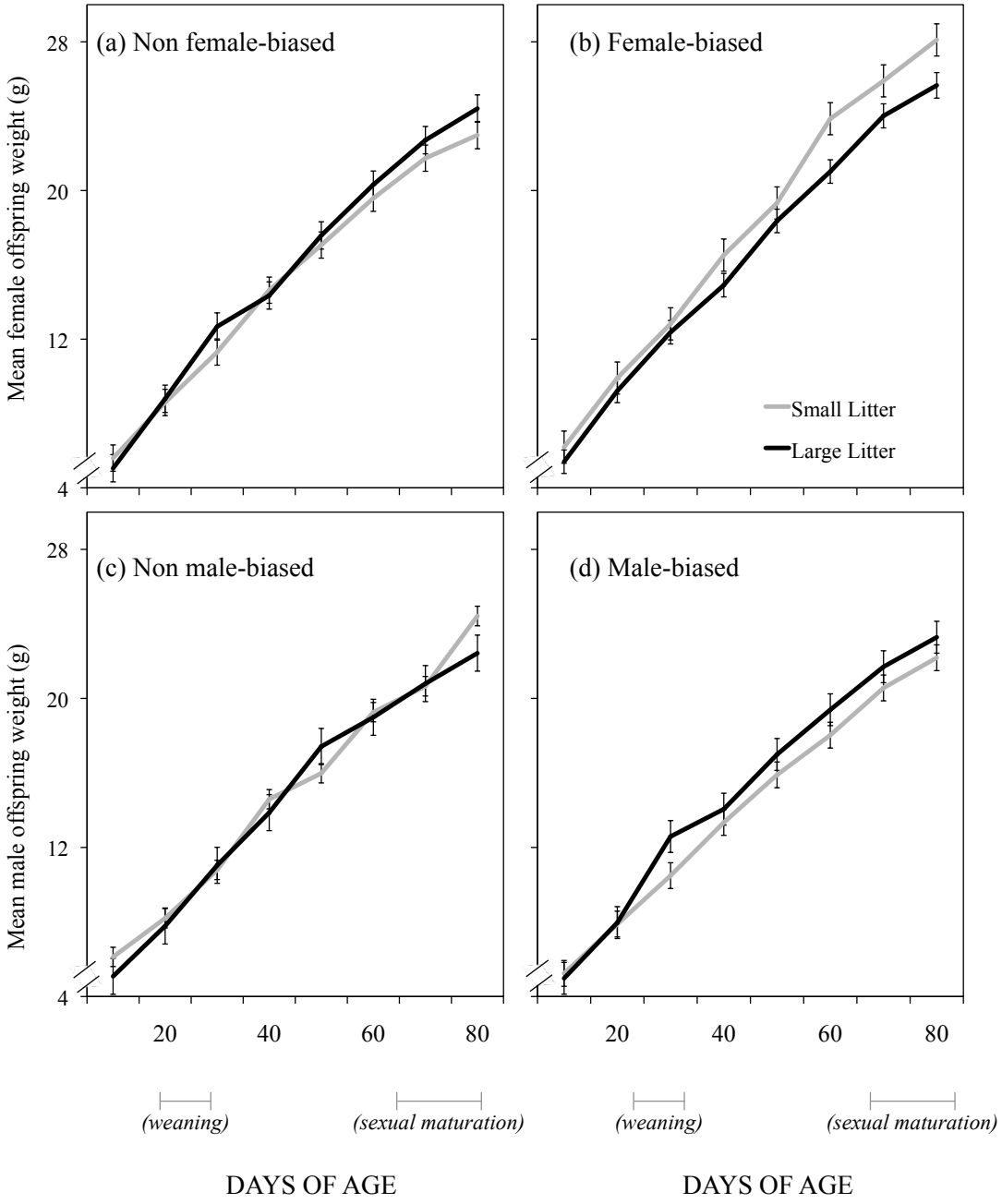

Figure 1. Growth of 27 female (top panels) and 35 male (bottom panels) pups reared either in litters biased toward their own sex (right panels) or in litters not biased toward their own sex (left panels). Means and standard errors are given for pups reared in litters with a small total birth-mass (grey lines) versus those with a large total birth-mass (black lines). Period of growth is measured in days of age along the horizontal axis. Twenty days of age is the start of the weaning period and 80 days is the end of the sexual maturation period.

litters obtain the heaviest weights by sexual maturity. An advantage in growth to reproductive age may or may not increase reproductive fitness. However, I demonstrated that the weight advantage due to having been born into female rather than male-biased litters does indeed result in a greater mean number of pups per litter for mature females. This suggests that it would be adaptive for female tarrkawarra to simultaneously adjust litter size and offspring sex to produce either small litters of female pups or large litters of male pups.

Maternal investment effects are increasingly well documented amongst mammals (see Cockburn et al. 2002; Grant & Chamley 2010 for reviews). Typically the quality of male rather than female offspring is the subject of these effects, reflecting sex-differentiated responses to developmental challenges. For example, male gerbil pups are more affected by periods of maternal absence than are female pups (Clark et al. 1990; Clark & Galef, 1991). Male pups are also typically reared in smaller litters and probably receive more milk than female pups indicating that gerbil mothers are sensitive to the greater demands of male offspring (Clark et al. 1990; Clark et al. 1991). A sex-role reversal in these early conditions, as experienced by tarrkawarra pups, may account for the findings here. Similarly, 80% of feral mares that experience a gain in condition around the time of conception produce sons (Cameron & Linklater 2007). Enhanced condition equips mothers for the extra costs of producing and rearing the larger sex offspring (although these costs are seldom evaluated). Consequently a bias to preferentially invest in sons, at least when maternal and environmental conditions are favourable, is typically expected and found in many male-biased SSD species (Koskela et al. 2004; Clark & Galef 1991). The mechanisms by which both litter size and sex

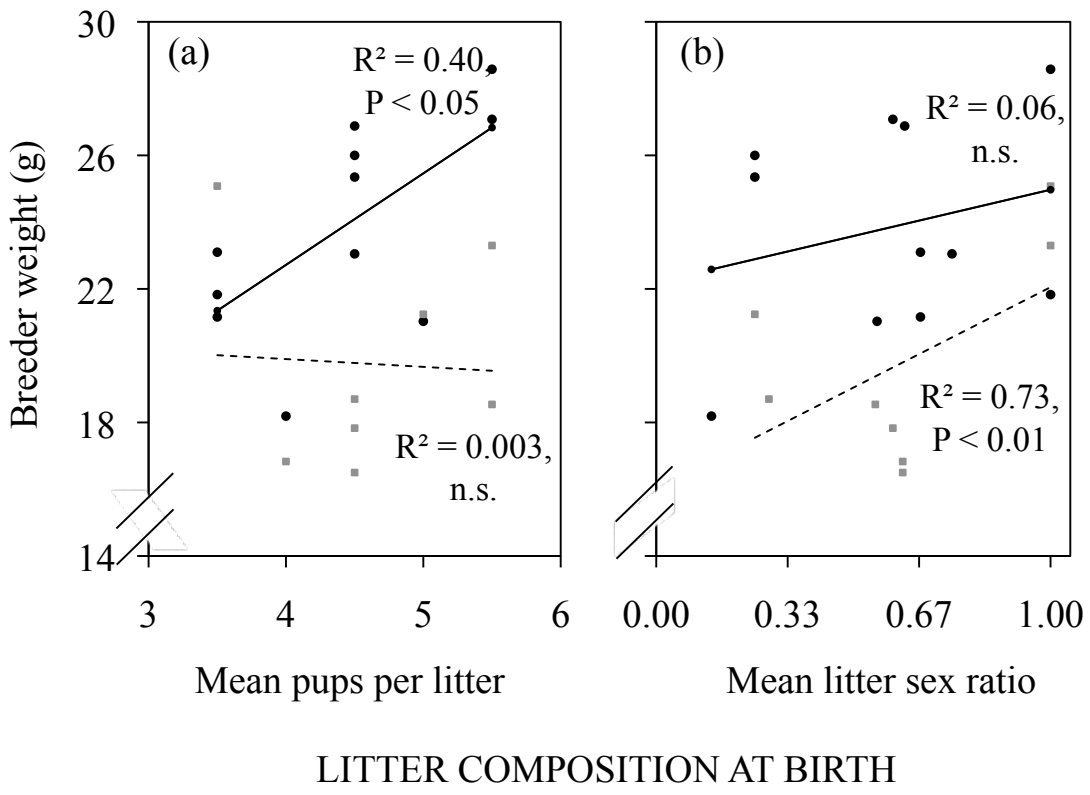

Figure 2. Mean weight at sexual maturity of dams (n=11, solid lines) and sires (n=8, dashed lines) by (a) the mean number of pups in the litters they subsequently produced and (b) the mean sex-ratio (male pup count / total pup count) of those litters.

ratio are adjusted are not clearly documented in many mammalian species to date but the studies that have investigated these phenomenon suggest that circulating levels of testosterone, glucose and cortisol immediately prior to and after conception may be important (Navara & Nelson 2009). Diet, stress and social conditions may all therefore impact on litter size and sex ratio adjustment.

The results presented herein clearly suggest a sex-biased investment pattern that contradicts those often found in male-biased SSD systems. Females in good condition (as gauged by weight) did not selectively invest in the larger-sized sex (females), as would be predicted, but did produce larger litters. It is interesting to note that the largest litters in this study (five pups) always had a male litter sex-ratio.

Sex-ratio effects that enhance female quality are found in some male-biased SSD mammalian systems. For example, greater maternal investment in daughters than in sons is indicated in both roe deer and small Japanese field mice when maternal condition is good (Hewison et al. 2005; Shibata & Kawamichi 2009 respectively) and may result in short-term FSSD during early development of roe deer (Hewison & Gaillard 1999). A similar reversal of the sex-ratio pattern typically expected in male-biased SSD species occurs regardless of maternal condition in bank voles, which are often FSSD (Bondrup-Nielsen & Ims 1990, Koskela et al. 2009).

The effect of litter sex-ratio on pup growth in this study cannot be attributed easily to maternal or environmental conditions as both food supply and housing conditions were standardised across the study. Post-hoc tests also suggested there was no significant correlation between individual pup and maternal weights. However, sex-ratio

effects may withstand variations in local conditions. For example, manipulating bank vole rearing-environments in the laboratory, via litter augmentation, does not alter maternal sex-biased investment and, as with tarrkawarra, male pup growth from birth to weaning is still significantly less than that of females (Koskela et al. 2009). The elimination of extraneous condition effects is important because, where maternal condition varies, sex-ratio effects may simply be a side effect of the relationship between maternal condition and maximum potential pup growth. If maternal condition is not controlled, the lesser weights of female pups from male sex-biased litters could be explained by the tendency of smaller females to produce smaller pups in general, regardless of whether they are female or male. This is clearly not the case in this study because it was the heavier rather than the lighter mothers that tended to produce the largest (and male sex-biased) litters. Additionally there was no tendency for male pups in female sex-biased litters to be larger (the converse effect).

In this study male tarrkawarra pup weights up to 80 days of age were not differentiated by either litter sex-bias or litter birth-mass. By contrast, growth is impaired in larger rather than smaller litters for male bank voles, male European rabbits and males of many other species (Koskela et al. 2009; Rödel & von Holst 2009 respectively). Male mammals are usually more sensitive to the local and maternal conditions that impact growth compared with females (Zuk 2009). Based on the findings here, a sex-reversal of this pattern may typify tarrkawarra offspring.

In male compared with female-biased litter sex ratios, female pups are more likely to occupy intrauterine positions (IUPs) beside a male or even between two males (Clark et al. 1993). Such IUPs are known to affect female sexual differentiation, probably via

greater exposure to testosterone, affecting a range of characteristics such as anogenital distance, duration to sexual maturation, the frequency of sexual behaviours and even the sex ratios of the litters females produce as adults (Ryan & Vandenbergh 2002; Clark & Galef 1995). Male adjacent IUPs and increased anogenital distances can ultimately also correlate with reduced litter sizes for female pups at maturation (domestic rabbits: Bánszegi et al. 2009; mongolian gerbils: Clark et al. 1993; house mice: Ryan & Vandenbergh 2002). In house mice and Mongolian gerbils these litters were also male-biased (Clark et al. 1993; Ryan & Vandenbergh 2002). As growth is part of normal sexual differentiation in tarrkawarra, it may be that females in non-female sex-biased litters are suffering the effects of male adjacent IUPs. The later effects of intrauterine position on an offspring's own litter composition in other species (Bánszegi et al. 2009; Clark et al. 1993; Ryan & Vandenbergh 2002) suggest a further sex-role reversal for tarrkawarra: large tarrkawarra females in this study tended to produce large litters (which were predominantly male-biased), despite the fact that litter composition does not affect male pup growth but does effect female pup growth (Chapter 2). Whilst I currently have no data on IUP effects or on post-natal sibling sex-specific competition in tarrkawarra, these phenomena certainly warrant further investigation as their effects alter the adaptive value of sex-ratio manipulation (Uller 2006).

Alternative hypotheses, specifically local mate and local resource competition hypotheses, may also explain why the heavier tarrkawarra dams in this study did not gear their reproductive efforts towards female offspring, instead producing large and typically male-biased litters. The former of these hypotheses suggests that sedentary species may attempt to reduce inbreeding amongst their offspring by over-producing daughters (Cockburn et al. 2002). Tarrkawarra are thought to be highly transient and

migrate over long distances (Dickman et al. 2010; Dickman et al. 1995), so local mate competition seems unlikely. However local resource competition, where the more dispersive sex is overproduced so as to reduce parent-offspring competition for forage or habitat (Cockburn et al. 2002), may bear consideration. By producing large litters of male pups, female tarrkawarra may reduce mother-offspring competition for local resources. This interpretation assumes a scramble competition system in the tarrkawarra, with small, mobile males dispersing long distances whilst females defend smaller home ranges. As the social organisation of tarrkawarra is not yet fully understood, this must be considered a plausible but tentative hypothesis (Chapter 2).

Our results show a direct fitness consequence of maternal investment (most likely via lactation) on tarrkawarra offspring. Specifically, by rearing daughters in small female-biased litters, tarrkawarra can increase these pups' weights at sexual maturity resulting in an average increase of 1.1 mean grand-offspring per litter, relative to other breeding females. By contrast, regardless of litter composition, mean grand-offspring per litter via sons, given equal mating opportunity, is steady. A similarly steady, size independent, rate of male reproduction is observed in the FSSD yellow-pine chipmunk (Schulte-Hostedde, 2004) and is attributed to stabilising selection on male size via opposing size advantages to running speed and dominance (Schulte-Hostedde, 2002). In both yellow-pine chipmunks and in tarrkawarra sex-differentiation of reproductive rates is opposite to that typically observed, where female reproductive rate is 'steady but sure', whilst a few males are disproportionately represented in the paternity of the subsequent generation (Rodríguez-Muñoz et al. 2010).

The findings presented here are essentially unique amongst the literature I am aware of, in demonstrating that litter sex-ratio can affect growth in a FSSD mammal (but see Bondrup-Nielsen & Ims 1990; in conjunction with Koskela et al. 2009). However, I did not observe a straightforward reversal of the well described 'quality sons/quantity daughters' scenario (Trivers & Willard 1973), commensurate with the 'reversed' direction of SSD in this species. Although female-biased maternal investment was suggested by the increased growth of female pups in female-biased versus non female-biased litters, investment in a greater number of male pups was associated with increased rather than decreased dam condition (as measured by body mass). This supports the argument that size and sex are not inter-changeable characteristics: it is important whether the larger sex is male or female (McDonald et al. 2005). Further data are needed to clarify the mechanisms of sex-biased maternal investment (*in utero* sex ratio adjustment, lactation variation etc.) and fitness consequences in tarrkawarra. In particular, paternally driven sex-biased investment is indicated by the finding that large males sire more sons. This suggests that larger male size in tarrkawarra either enhances specific forms of sperm performance or that a heritable component to size is coupled with a female preference for larger over smaller males. Alternatively, if smaller male tarrkawarra are more attractive, female investment may increase (larger, male biased litters) to compensate for having offspring sired by an unattractive (large) mate (Ratikainen & Kokko 2010).

In summary, female-biased maternal investment seems highly plausible in this tarrkawarra and likely to reflect a fitness advantage of larger adult female size. The male-biased maternal investment by larger mothers is more difficult to explain and requires further investigation, ideally utilising the natural ease with which this species

cross-fosters pups. Given the clear advantage to female pups of being reared in female sex-biased litters, and assuming full maternal control of litter sex-ratio, it also remains to be explained why dams suffer the fitness cost of mixed sex litters.

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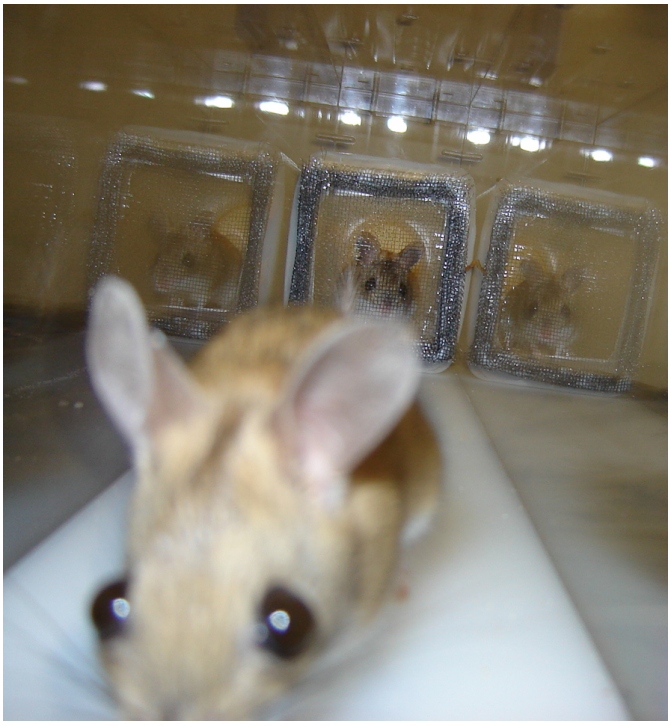
Chapter 4

Can female mate-choice explain female-biased sexual size dimorphism in tarrkawarra (*Notomys alexis*)?

In preparation for submission to *Animal Behaviour*



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ABSTRACT

Female-biased sexual size dimorphism (FSSD) in mammals is poorly represented in the empirical literature and inadequately explained by theory. In particular the contribution of sexual selection in driving size differences, has been overlooked despite its importance in non-mammalian FSSD taxa. I investigated whether female mate choice in the FSSD mammal tarrkawarra (*Notomys alexis* or spinifex hopping mice) reflects selection for smaller males, thereby contributing to small male size relative to female size. I compared size-based and familiarity-based association preferences of captive females, when sexually receptive and when non-receptive. All females showed a significant preference for relatively larger males when non-receptive but once receptive, only experienced females continued to discriminate based on size. However, irrespective of receptivity, experienced females failed to discriminate between males based on familiarity whilst inexperienced females significantly preferred unfamiliar males. Female - male results were qualitatively very different from female – female association preferences and were characterized by 'darting' behaviour, a pre-copulatory sequence not previously recorded in this species. Specifically, receptive females increased their visit frequency (darting) in response to males but not females whilst the corresponding sum duration of visits did not change significantly in response to either sex. The strong evidence for a large male advantage based on female preference, makes the persistence of FSSD in this species even more puzzling.

INTRODUCTION

Female choosiness over mates is a mechanism by which female fitness can be increased despite limitations imposed by egg production, gestation or incubation, lactation or offspring provisioning (Bateman 1948; Jones et al. 2005). Production of high-quality, highly fecund offspring offset these restrictions and female choice may reflect ways that paternity can facilitate this (e.g. genetic superiority, additional parental care, access to preferred territories, see Andersson & Simmons 2006). For example, when body size is highly correlated with condition and ability to defend resources, females often show a preference for larger males (Andersson 1994). Female choice may thereby facilitate the evolution of male-biased sexual size dimorphism (SSD), particularly when accompanied by strong selection on male size via male-male competition (Blanckenhorn 2000). However, in most species other than mammals, female-biased sexual size dimorphism (FSSD) is the norm (Fairbairn 1997).

In non-mammalian taxa, FSSD is apparently the product of strong fecundity selection for large female size despite levels of male-male competition commensurate with those seen in mammals (Blanckenhorn 2000). Fecundity selection for large female size is also prevalent in mammals and arguably no more marked in FSSD than non-FSSD species (Eens & Pinxten 2000; Bonduriansky 2001; Lammers et al. 2001; Schulte-Hostedde et al. 2001; Bornholdt et al. 2008; but see also Lindenfors et al. 2007). Fecundity selection is therefore insufficient by itself to explain instances of mammalian FSSD.

Mammalian FSSD theory typically excludes sexual selection as a significant selective agent on the assumption that FSSD mammals are characterised by low male-male competition and an absence of polygyny (Isaac 2005; Ralls 1976; Schulte-Hostedde et al. 2004). Hypothetically this relaxes selection on male size. However, it does not adequately explain why a) male-male competition and polygyny are reported in a number of some FSSD mammals (e.g. humpback whales Spitz et al. 2002; greater sac-winged bats Voigt et al. 2005; Weddell seals Harcourt et al. 2007; crabeater seals and dugongs Ralls & Mesnick 2008) and b) why males are smaller rather than equal in size to females. Divergence of sexual morphotypes from a shared genome is costly and opposite selection on both sexes is presumably required for SSD to evolve (Fairbairn 1997). Consequently it seems highly likely that mammalian FSSD must be explained, at least in part, (as it is in other taxa – see Elgar et al. 2000; Moya−Laraño et al. 2002; Kasumovic & Andrade 2009) by a negative correlation between male fitness and size (Blanckenhorn et al. 1995; Blanckenhorn 2000). A females' preference for smaller males would ensure these benefits are passed on to her sons. It is therefore surprising that female mate choice has not previously been investigated in FSSD mammals.

Although the literature predominantly records female preferences for larger males, instances where sexual selection favours smaller males are by no means uncommon (Blanckenhorn 2000). For example, female moorhens actively compete to mate with smaller (albeit fatter) males (Petrie 1983). Likewise, relatively smaller orb web spider males enjoy a greater paternity than their larger rivals in double mating trials (Elgar et al. 2000). In fact evidence for small-male sexual selection exists in 21 spider species (summarised in Blanckenhorn 2000). In water striders, female mate-choice (or rather mate-guarding tolerance) also favours smaller males and male body size is negatively

correlated with mating success (Sih et al. 2002). However counter-selection on larger male appendages that combat female mating resistance ultimately results in slightly greater fitness in larger over smaller males (Blanckenhorn et al. 1995). Clearly female mating preferences and male traits under selection for reproductive success can be at odds with each other.

There is only one example from the mammalian FSSD literature that is suggestive of a small-male bias in female mate choice. In the FSSD greater sac-winged bat, aerial courtship and harem defense behaviours select for smaller males and male paternity within harems increases as male size decreases (Voigt et al. 2005). The apparent rarity of sexual selection in FSSD mammals may, as is commonly assumed, be because mammals are subject to different fitness consequences and constraints on size than other taxa (Andersson 1994). Alternatively it may simply reflect study species biases that have hitherto excluded mammals in which small male advantages seems most likely – that is FSSD species.

In the FSSD tarrkawarra (*Notomys alexis* or spinifex hopping mouse), sexual selection on smaller male size is predicted assuming increased fitness of smaller males in scramble competition (Blanckenhorn 2000). Scramble competition is indicated in this species for several reasons: there is an absence of behavioural or sperm competition between males (Breed & Ford 2007); trapping patterns indicate high transience (Dickman et al. 2010; Dickman et al. 1995); and the habitat is highly variable in its ability to support large numbers of animals (Letnic & Dickman 2006). Selection under scramble competition hypothetically favours smaller males whose lower energetic requirements free them up for more efficient mate searching (Blanckenhorn 2005).

Relatively high levels of FSSD have been found to relate to environmental 'severity' in chipmunks (Levenson 1990). In addition to selection for scramble competition, the similarly harsh and often food-limited habitat of the tarrkawarra (see methods) may further select for small male size. At least at times of low population density (the majority of inter-generational time), small tarrkawarra males may be better prospects for females than larger starvation-prone and forage-hungry males.

Here I investigated whether female mate choice in the FSSD mammal tarrkawarra reflects selection for smaller males. Because the mating system of tarrkawarra is not fully known and may be flexible depending on population-density (Bradley 2009; Dickman et al. 2010), I assessed female mate choice, not just based on the size of potential mates, but also based on their familiarity to the female. I predicted that, whilst sexually receptive, females would preferentially associate with smaller males reflecting fitness consequences of smaller male body size that accrue to offspring.

METHODS

Tarrkawarra are a semi-fossorial FSSD rodent endemic to the central arid zone of Australia where boom and bust conditions prevail. They are morphologically similar to other desert dwelling hopping species in North America, Africa and Asia (e.g. kangaroo rats, gerbils and jerboas Randall 1994). Receptive females mate multiply and alternate paternity of whole litters between available males but there is no evidence to date for mixed litter paternity (Breed & Adams 1992). Despite limitations in their reproductive physiology (Breed & Sarafis 1979; Breed 1980, 1981; Kenagy & Trombulak 1986; Suttle et al. 1988; Breed et al. 1990; Peirce & Breed 1990, 2001; Peirce et al. 2003)

males can mate successfully with several females within days of each other, suggesting that they compensate for small sperm reserves by rapid reserve replenishment (Bauer & Breed 2008).

The tarrkawarra oestrus cycle lasts six to nine days (Smith et al. 1972; Crichton 1974; Breed & Papps 1976) although it may reduce after the first year of life (Telfer & Breed 1976). Ovulation is spontaneous, regardless of whether males are present or otherwise (Breed 1975) and full oestrus typically lasts for one to two days (Crichton 1974) and up to four days (pers obs.). Cyclic changes can be divided into three stages and identified from vaginal smears as follows (after Breed 1975): pro-oestrus (nucleated epithelial cells prevail, few if any leucocytes), oestrus (cornified cells prevail, no leucocytes), and dioestrus (leucocytes prevail, few nucleated epithelial or cornified cells). Tarrkawarra have a very short pro-oestrus phase and vaginal smears in this study often indicated borderline oestrus (a mixture of nucleated epithelial and cornified cells). Although sexual receptivity is usually associated only with oestrus, I combined pro-oestrus and oestrus data for analysis purposes into a 'receptive' category to contrast with 'non-receptive' dioestrus data.

Animal Housing

I allocated sixteen female tarrkawarra, all born and reared in a captive colony at Macquarie University, Sydney, Australia, into eight pairs. These focal females varied in terms of their body mass, age and as to whether they were sexually experienced or inexperienced (Table 1). Each pair of focal females was allocated two pairs of unique and wholly unfamiliar potential mates (males) or affiliates (females) (Table 2). Minimising relatedness with focal females, I selected these eight potential mates and

eight affiliates from outbred colony animals. Mate and affiliate pairs were the smallest and largest of same-sex siblings available. This maximised the relative size differences of potential mate or affiliate combinations possible during subsequent testing. Apart from the use of female animals, affiliate trials were run in an identical manner to mate trials and acted as a control group in the analysis, allowing for comparison of reproductive (mate) versus social (affiliate) preferences.

Table 1. Age and weight of focal females (those choosing between potential mates or affiliates) by sexual experience

	Focal females (N = 16)			
	Inexperienced†	Experienced‡	t_{14}	P
Mean weight* (g) ± SE	39.1 ± 0.7	40.5 ± 0.3	-0.46	0.65
Weight range (g)	26.6 – 53.3	34.9 – 49.3		
Mean age (month) ± SE	19.2 ± 0.3	19.5 ± 0.2	-0.42	0.68
Age range (month)	13.3 – 23.5	13.1 – 23.6		

**Mean of weights taken daily to the nearest 0.01g.*

†Virgin females, single-sex housed post weaning.

‡Females that had matings or mating opportunities but no offspring.

Note that the maximum female weights in this sample are only observed in pregnant females in the field, suggesting that the captive conditions here may be promoting unnaturally high body mass.

Animals were housed in solid-bottom polycarbonate cages (50 × 40 × 40 cm, L × W × D) with paper pellet substrate, tissue bedding and constant access to water and food (millet seed mix and fresh carrot cubes). I maintained the colony at 20-23°C, on a 14:10 h light:dark cycle with lights off at 1400 and ran experiments within the first three hours of the dark phase. Housing arrangements varied across the course of the experiment so that both designated pairs of potential mates or affiliates (Table 3) could be presented to focal female pairs initially as unfamiliar animals and subsequently again as familiar animals. Familiarity between previously unfamiliar pairs of animals was

achieved, when required, through a comprehensive familiarisation process. Firstly I swapped animals into adjoining cages each day shortly before the start of the dark phase in the reversed cycle lighting schedule. Bedding and substrate was not changed over the eight-day familiarisation period and this provided females with access to olfactory cues from non-focal animals for one full oestrous cycle. Providing olfactory stimuli has traditionally been regarded as critical in the familiarisation process between rodents but it may not be sufficient for fully integrated multi-modal identification of individuals.

Table 2. Age and weight of potential male mates and female affiliates

	Female affiliates (N = 8)	Male potential mates (N = 8)
Mean weight* (g) ± SE	41.2 ± 0.6	31.0 ± 0.4
Weight range (g)	26.8 – 53.6	24.3 – 38.9
Mean age (month) ± SE	20.2 ± 0.2	16.5 ± 0.3
Age range (month)	13.1 – 23.5	10.8 – 24.2

**Mean of weights taken daily to the nearest 0.1g.*

Using through-mesh exposures, Johnston and Peng (2008) demonstrated in hamsters that familiarisation procedures with a physical contact component are much more effective than simple odour habituation procedures. Therefore, I also included a physical contact component in my familiarisation procedure by linking home cages of pairs to be familiarised with two 80mm PVC pipes. Animals could then transit through these pipes, throughout the eight-day familiarisation period, and interact with animals in the neighbouring cage through a wire mesh grill affixed at the other end. Mesh gage openings were 1cm^2, sufficient to allow animals to contact one another with noses and paws. Animals frequently sniffed, physically contacted and vocalised loudly to one another through the grill, especially in the first few days of the familiarisation period. Animals under the conditions described above were deemed familiar after eight days -

one full oestrus cycle. In the weeks before and during the experiment, vaginal smears (as a well-established indirect measure of the presence or absence of behavioural oestrus) of all female animals were analysed daily and categorised as 'receptive' or 'non-receptive' for later analysis (see above).

Table 3. Familiarity status of non-focal animals on each day they were presented to focal females in the apparatus

		Non-focal animal / potential 'mate'			
Trial	Day	A	B	C	D
1	†1	Unfamiliar	Unfamiliar	-	-
2	8	Familiar	Familiar	-	-
3	10	-	-	Unfamiliar	Unfamiliar
4	12	Familiar	-	Unfamiliar	-
5	14	-	Familiar	-	Unfamiliar
6	16	Familiar	-	-	Unfamiliar
7	‡18	-	Familiar	Unfamiliar	-
8	26	-	-	Familiar	Familiar

†Home cages reconfigured to familiarise focal females with A and B immediately after trial.
‡Home cages reconfigured to familiarise focal females with C and D the following day.

Study Design & Procedure

I conducted eight two-choice trials with each focal female. Oestrous cycling was not manipulated and trials occurred opportunistically at different stages of the female's oestrous cycles. Choices were either always between potential mates or between affiliates. In a repeated measures design, each of four non-focal animals (mates or affiliates) was presented at least once as alternatives to each of the others. Each non-focal animal was also presented, at least once, both prior to and after familiarisation with the focal animal and the trial run order ensured that all familiarity combinations were assessed (Table 3). Additionally, although the size difference between non-focal

options varied from minimal to substantial, only two males were never in trials against a larger male and only one was never in a trial against a smaller male. At the start of each trial, focal females were transported from their home cages into the test room. They were released from transporter boxes into the open topped 'hub' of the apparatus. They spent 30 mins in the apparatus and were free to enter, exit and re-enter three lidded arms radiating from the hub (Fig. 1). At the end of two of these arms, a non-focal animal was occupant in a 10 × 15 × 20 cm (L × W × H) space, behind a mesh grill. The mesh was wide enough gauge to allow tarrkawarra to interact physically using paws and nose. The centrally located arm was unoccupied and was included to control for any preference for enclosed space. Side placement of non-focal animals in the apparatus was balanced across trials for familiarity with the focal female and relative size compared with the other non-focal animal in the apparatus during the trial.

Table 3 details the experimental run order according to the familiarity status of the four non-focal animals on any given trial. On the first trial focal females were unfamiliar with both non-focal animals. Housing to produce familiarity between the focal and non-focal animals was implemented later the same day. After the familiarisation period (eight days), six trials were run every second day with each possible combination of the now familiar non-focal animals and a second, unfamiliar, pair of non-focal animals. Trial order was randomised. Home cages were then reconfigured to produce familiarity between the focal female and the second, still unfamiliar pair of non-focal animals. Eight days later these animals were deemed familiar and final trials were run.

Trials were observed and filmed remotely under infrared lighting, with a Sony DV Camera (Model DCR-TRV355E PAL). Scoring of footage was done by observers blind as to the familiarity, relative size and reproductive state of all animals. Observers produced an observation log for each occupied arm, from which I subsequently calculated visit frequency to within one body length of the grills and sum duration of focal female visits. In total there were 256 observations - half from trials with male potential mates and half from trials with female affiliates. The two blocks of 64 trials were analysed separately using a within and between subjects model.

Statistical Methods

An SPSS mixed linear model procedure was used. This allowed for a multilevel model with three levels: individual females, trials within females, and two observations within each trial. The mixed model took into account the dependence between trials for the same animal, and responses within trials. Animals and trials made up the random part of the model. One advantage of this approach was that it was not necessary to calculate ratios or difference scores, both of which can be problematic as dependent variables (Cohen et al. 2003). Four fixed factors, each with two levels, made up the fixed part of the model: focal female sexual experience (experienced or inexperienced), focal female receptivity (receptive or non-receptive), potential mate or affiliate familiarity (familiar or unfamiliar to the focal female) and potential mate or affiliate size (larger or smaller relative to the other non-focal animal in the apparatus on that trial). Data were log 10 transformed to account for skew where necessary. Stepwise backward reductions of non-significant terms were also preformed but did not explain the data any further.

Ethical Note

The research described in this paper was approved by the Macquarie University Animal Ethics Committee (protocol number: 2005018) and is in accordance with the ASAB Guidelines for the use of Animals in Research.

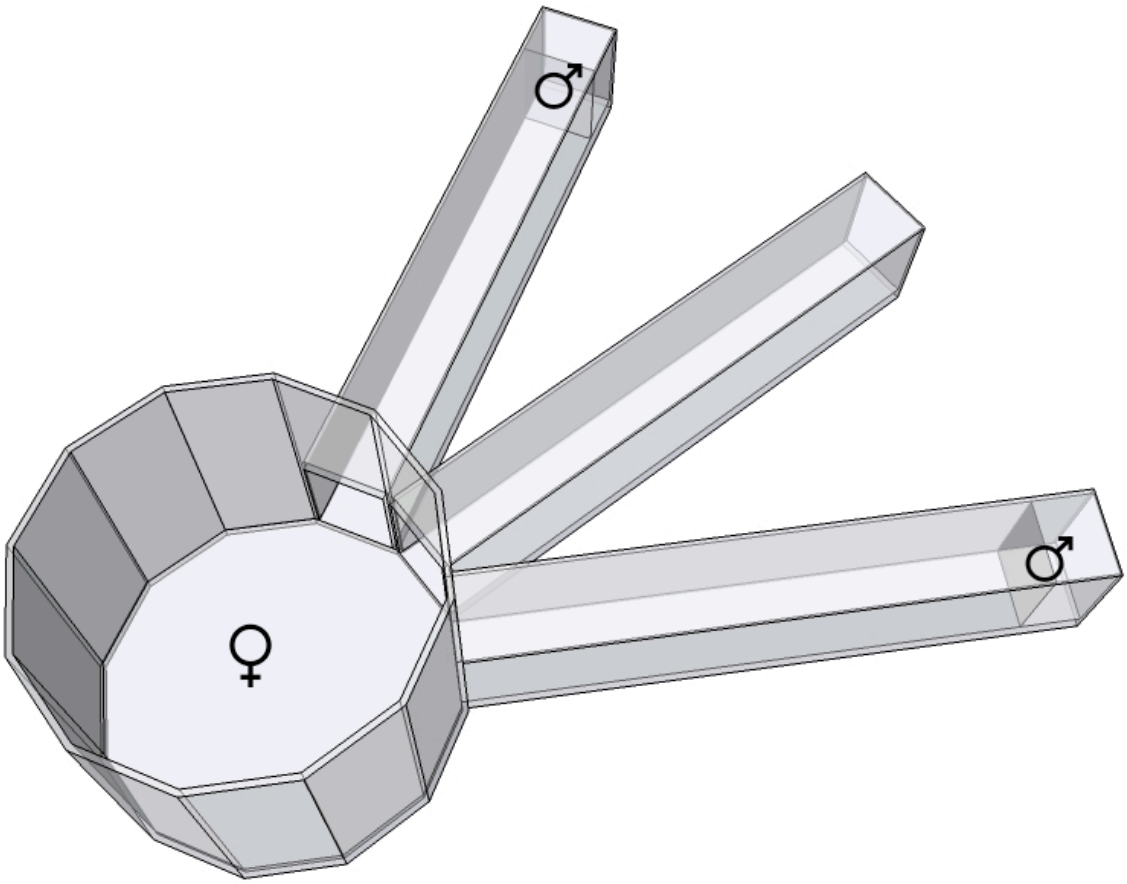

Figure 1. Overhead view of the mate choice apparatus. Three closed-top arms measured 100 × 15 × 20 cm (L × W × H) and could be entered from the circular, open-top hub, measuring 50 × 60 cm (H × diameter). Wide gauge mesh grills, 10 cm from the closed ends of the two outer arms, separated focal from non-focal animals during trials.

RESULTS

Altogether sixteen focal females were tested with four non-focal individuals each – either four male potential mates or four female affiliates. There was an opportunity for each non-focal individual to be compared with each of the other three, and an opportunity for each non-focal individual to be assessed both prior to and after familiarisation with the focal animal. In moving around the apparatus, focal females usually entered both occupied arms, and interacted with the non-focal animals through the grills within the first minute. In fact initial sampling often occurred much sooner than this and, throughout the mate trials, I also observed frequent bouts of high speed 'shuttling' behaviour, with females moving rapidly in and out of a single occupied arm and/or between occupied arms. This behaviour clearly represented a very different type of 'visiting' behaviour compared with total time spent fixed alongside the non-focal animals. The shuttling behaviour observed is characteristic of a chase initiation sequence known as 'darting', a pre-copulatory behaviour seen in other female rodents (Barnett 1976; Burley 1980; Sachser et al. 1999). Visit frequency (total number of focal female transits to within one body length of grill) was therefore analysed as the main dependent variable, in addition to the more traditional visit duration measure (sum total seconds that focal females were located within one body length of the grills). Focal females spent negligible amounts of time in the central, unoccupied arm and data from these visits were not included in the analysis.

Focal Female Visiting of Male Potential Mates

Using the duration data, I first applied the model described above to address the question of whether focal females spent more or less time with males based on male size and familiarity and whether this varied according to the female's own sexual experience and receptivity. None of the three-way or the four-way higher order effects were significant ($P > 0.05$) and so these effects were removed from the model in a stepwise progression, followed by two-way interactions wherever $P > 0.05$. The final model retained all main order effects and one two-way higher order effect (focal female sexual experience by male size).

Next I applied the model to the visit frequency data to determine how male size and familiarity and female sexual experience and receptivity affected female visit rates. As with visit duration, the non-significant four-way and three-way higher order effects were removed from the model, in a stepwise progression. Ultimately only one three-way higher order effect remained (female receptivity by female sexual experience by male size). All non-significant two-way interactions not incorporated in the three-way interaction were then also removed from the model in a stepwise progression. The final model retained all main order effects, five two-way and one three-way higher order effect (Table 4).

Overall (and in most interactions), visit duration trends were reflected in visit frequency data. A trade off between visit frequency and visit duration, however, reduced any visit duration effects to below statistical significance. Importantly, at the critical time for assessing mate choice (when females are reproductively receptive), visit duration was not different from non-receptive periods ($F_{1,136.4} = 0.027$, $P = 0.87$; Fig.

2a), whilst visit frequency was significantly elevated compared with when females were non-receptive (Fig. 2b; Table 4). If conclusions about reproductively motivated versus non-reproductively behaviour are to be drawn from association behaviour (such as that measured here), then it is important the dependent variable is sensitive to changes in behaviour when females become sexually receptive compared with when they are non-receptive. Consequently, I continued analysis on visit frequency data only and made no further analyses of visit duration data.

Table 4. Effects of female sexual experience and sexual receptivity on visiting frequency to familiar versus unfamiliar and larger versus smaller males

Fixed effects*	*F*	*P*
Male familiarity	$4.37_{1, 122.8}$	0.039
Male size	$15.18_{1, 66.8}$	0.000
Sexual experience (of focal female)	$0.93_{1, 6.2}$	0.371
Sexual receptivity (of focal female)	$13.37_{1, 62.2}$	0.001
Male familiarity by sexual experience	$4.38_{1, 121.7}$	0.038
Male familiarity by sexual receptivity	$0.45_{1, 122.9}$	0.502
Male size by sexual experience	$1.54_{1, 65.6}$	0.218
Male size by sexual receptivity	$4.04_{1, 67.9}$	0.049
Sexual experience by sexual receptivity	$2.30_{1, 62.2}$	0.135
Sexual experience by sexual receptivity by male size	$4.04_{1, 68.1}$	0.048

**Data were analysed using a mixed linear analysis and log10 transformed to correct for skew.*

The ongoing analysis of visit frequency data yielded further main effects for male familiarity and size with focal females visiting unfamiliar and larger males significantly more often than familiar and smaller males (Table 4).

Although there was no main effect of focal female sexual experience it was a significant determinant of visit frequency in combination with other factors. Receptive

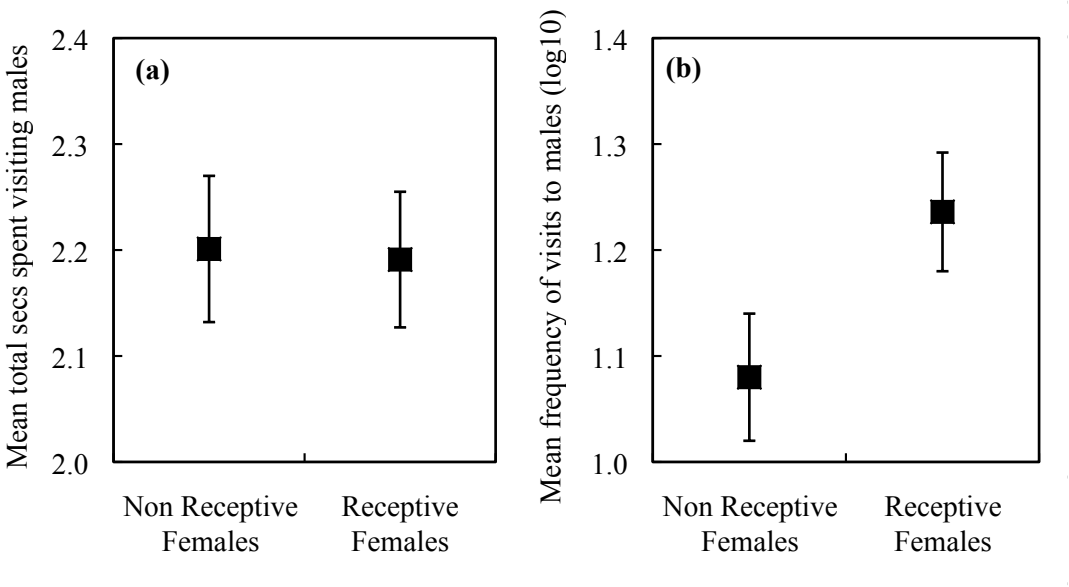

Figure 2. Association of sexually receptive versus sexually non-receptive focal females with potential male mates as measured by (a) mean sum seconds duration (±SE) spent within one body length of grill (n.s.) and (b) mean frequency (±SE) of female visits to grills irrespective of time spent once there ($P < 0.01$).

focal females visited larger males significantly more often than smaller males (Table 4) and notably more often when those females were also sexually experienced (Table 4). By comparison their inexperienced counterparts did not clearly differentiate at the critical point in their reproductive cycles, and visited larger rather than smaller males more often only when non-receptive (Fig. 3).

Finally, and independent of the three-way interaction, the effect of focal female sexual experience also varied with male familiarity (Table 4). Tests of simple effects showed that whilst sexually experienced focal females did not differentiate between males based on familiarity alone, inexperienced focal females visited unfamiliar males significantly more often than familiar males (Fig. 4).

Focal Female Visiting of Potential Female Affiliates

To test whether the effects described above can be legitimately attributed to choice between mating alternatives rather than affiliative preferences, data from the observations where focal females were presented with two non-focal females were also analysed for effects of familiarity, relative size, focal female sexual experience and receptivity. The reduced mixed linear models described above were used and no significant effects were found for visit frequency (P values ranged from 0.162 – 0.925). In contrast to their behaviour with male potential mates, however, focal females spent more time visiting familiar than unfamiliar females ($F_{1,132.01} = 4.963$, $P = 0.028$) further suggesting that visit frequency and visit duration are not directly comparable measures in regards to mate choice for this species.

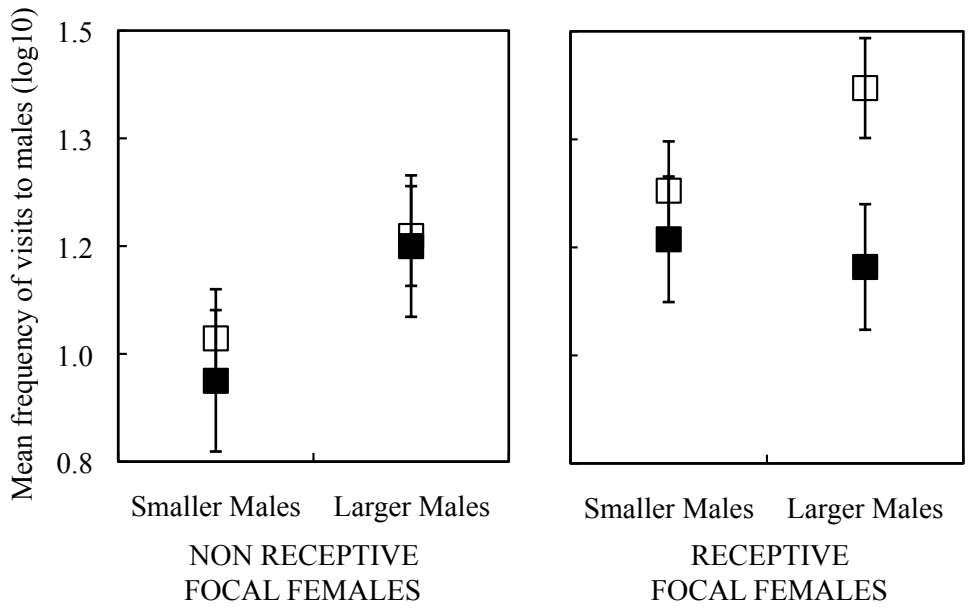

Figure 3. Visit frequency of sexually non-receptive and (left panel) sexually receptive (right panel) focal females to males, as a function of female sexual experience and male size. Open squares indicate sexually experienced female means (±SE) and closed squares indicate sexually inexperienced female means (±SE). Tests for simple effects gave significant results for all comparisons of means (*P*s < 0.0001 - 0.05) except for experience-dependent non-receptive female responding to smaller males ($F_{1,12.1}$ = 0.24, P = 0.63) and to larger males ($F_{1,12.1}$ = 0.01, P = 0.91), experienced-dependent receptive female responding to smaller males ($F_{1,8.9}$ = 0.37, P = 0.56), inexperienced receptive female responding to smaller versus larger males ($F_{1,68.4}$ = 0.51, P = 0.477) and receptivity-dependent responding of inexperienced females to larger males ($F_{1,112.7}$ = 0.12, P = 0.733).

DISCUSSION

In this study the best 'primed' females (those that were both sexually receptive and experienced), clearly preferred larger over smaller males. This preference was consistent even when females were not physiologically ready for mating (i.e. non-receptive). However, it is the response whilst receptive that most strongly suggests that the large-male association pattern reflects a genuine mating preference. Supporting this is the lack of any size discrimination in response to female affiliates and the qualitative differences between the behaviours directed at females versus those directed at males. Specifically, receptive focal females darted back-and-forth to males in a sequence that is known to precede copulation in other rodents (Barnett 1976; Burley 1980; Sachser et al. 1999) and they did not do this in response to female affiliates. Surprisingly, however, given their strong size preference, sexually experienced females were indiscriminate based on the familiarity of males although inexperienced females preferred unfamiliar over familiar males. Although, female familiarity-discrimination of males did not vary with receptivity, they can be cautiously interpreted in terms of mate choice given the contrasting responses of focal females to the same variables in female affiliates.

A large-male preference is a fairly typical finding in non-FSSD mammals but in non-mammalian FSSD taxa this preference is sometimes reversed (Blanckenhorn 2000; Elgar et al. 2000; Moya−Laraño et al. 2002). However, response rates of both receptive and non-receptive tarrkawarra females in this study were at their lowest in the presence of small males. It is therefore problematic to attribute FSSD in this species to sexual selection on small male size. Pitted against larger competitors, a small tarrkawarra male

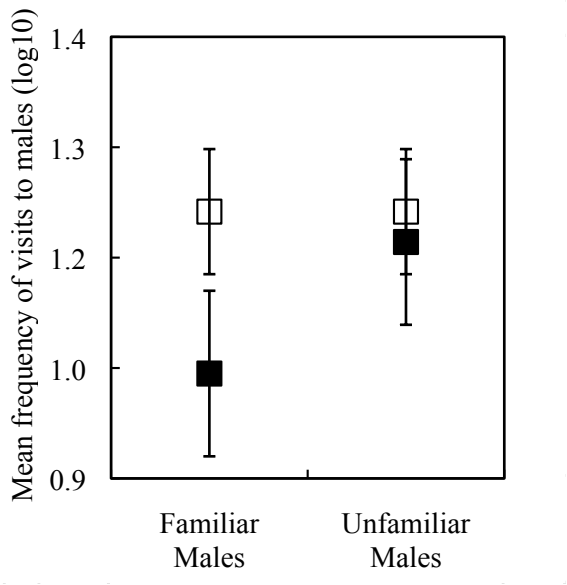

Figure 4. Frequency of focal female visits to familiar versus unfamiliar males as a function of female sexual experience. Open squares indicate sexually experienced focal female means (±SE) and closed squares indicate inexperienced focal female means (±SE). Contrary to appearances, tests of simple effects indicated that the experience-dependent difference in focal female responding to familiar males is non-significant ($F_{1,7.5}$ = 2.40, P = 0.16). Only the familiarity-dependent difference in responding of inexperienced focal females was significant in this interaction ($F_{1,121.2}$ = 6.52, P < 0.05).

seems unlikely to achieve many fertilisations unless there is a high incidence of less choosy, inexperienced females and/or if larger preferred males are sperm depleted. These conditions may, however, prevail during high-density population events (Wedell et al. 2002; Kokko & Mappes 2005; Bro-Jørgensen 2007). Small male genes may persist through periods where large male reproductive success is greatest, until selection on smaller phenotypes (via predation avoidance, starvation resistance etc.) is critical once again (see Bisazza & Pilastro 1997 for a similar scenario in *poeciliidae*; and Higginson & Reader 2009 regarding genotype-environmental interaction effects on sexual selection).

Selection of males, based on familiarity, may vary depending on local conditions experienced by the female. For instance, a preference for unfamiliar opposite sex associates is typical of non-monogamous species, whereas familiar partners are usually preferred in pair-living species (Salo & Dewsbury 1995). In this study the relatively low frequency of inexperienced female visits to familiar males indicates that females sampled these males only minimally. They darted significantly more in response to unfamiliar males. By contrast, in response to female affiliates that differentiated on familiarity, focal female visit rates were not significantly different. However they spent more time with familiar than unfamiliar female affiliates and this was the only distinction they made between female affiliates (significant main effect). One interpretation of this sex-specific response is that a preference for unfamiliar over familiar males in mate-choice contexts represents inbreeding avoidance. Such mechanisms are characteristic of species in which related individuals do not disperse prior to their first breeding effort (Pusey & Wolf 1996). On the other hand, virgin females have also been hypothesised to be indiscriminate in anticipation of future

mating opportunities in which to compensate for poorer quality offspring produced during the current breeding attempt (Kokko & Mappes 2005). For non-virgin females, however, previous successful reproductive efforts may decrease the fitness cost of producing few or poor quality subsequent offspring and, in turn, also make older, parous females less choosy (Jennions & Petrie 2000). Typically, in mate choice experiments, such as the one presented here, female 'experience' is based on parity (having produced offspring) and is also often correlated with age. Choosiness may reduce as fecundity and reproductive opportunities dwindle with age (reproductive senescence Moore & Moore 2001; Kodric−Brown & Nicoletto 2001; Richard et al. 2005). However, in this study, inexperienced (virgin) and experienced (housed with males but unsuccessfully mated) females did not differ in mean age and all were non-parous. This suggests that sexual experience alone may be enough to affect mate choice behaviour regardless of age or parity.

A lack of pre-copulatory choosiness does not necessarily imply absence of mate selection. In some species poor quality mates may be eliminated prior to fertilisation through sperm competition or cryptic female mate choice (Andersson & Simmons 2006). Sperm competition has been considered unlikely in tarrkawarra due to a low testes:body mass and profoundly diminished spermatozoa production, storage and transport capability relative to other mammals (Breed et al. 1990; Breed & Sarafis 1979; Breed 1980, 1981; Kenagy & Trombulak 1986; Suttle et al. 1988; Peirce & Breed 1990, 2001; Peirce et al. 2003). Under these conditions, preferred males presumably quickly become sperm depleted whenever reproductively active females are spatially clustered (as in times of high population density) (Warner et al. 1995; Bro-Jørgensen 2007). However a recent finding that males can rapidly replenish sperm reserves and

successfully fertilise several females within a matter of days (Bauer & Breed 2008) re-opens the case against some of these assumptions. The finding here that females are choosy, further suggests that tarrkawarra sperm may not be as limiting a resource as previously thought.

Assessing female mate-choice using association behaviour is notoriously fallible as association indices are imperfect predictors of actual mating tendency (Walling et al. 2010). However, it is not always ethically acceptable to run assays that eventuate in copulations and surplus animals. Fortunately, both quantifiably and observationally, receptive tarrkawarra females in this study traded off time spent with males against time spent running rapidly towards and away from them, in a characteristic 'darting' pattern. In rats, gerbils and some cavies the darting sequence is described as 'solicitous' because it activates male pursuit, mounting and copulation behaviours (Barnett 1976; Burley 1980; Sachser et al. 1999) and precedes intromission in as many as 90% of cases (Ågmo 1999). Although its precedence to copulation must still be validated, this makes darting a very promising assay of female mate choice in tarrkawarra.

One reason darting may not have previously been noted in tarrkawarra is that, once sexually experienced, unconstrained males are rapidly responsive to receptive females regardless of whether they dart or not (Ågmo 1999). This effect of male-experience, combined with the fact that polyandrous females do not always mate with preferred males first, may actually render copulation-based assays of mating preference less reliable for species such as these (Burley 1980). The design presented here ensured that I obtained both female preference functions and a measure of choosiness. The former indicates the direction or absolute rank of compared mate variables and is

typically all that can be determined from conventional two-choice tests (Wagner 1998). However, by comparing darting frequencies directed at both males over repeated trials, I also obtained a measure of the change in willingness (or 'choosiness') of individual females to compare mating options in different situations (Jennions & Petrie 1997). Nevertheless, it should be noted that non-receptive females in general, in this study, also appeared to solicit larger males relatively more often than smaller males. This is surprising because solicitous darting behaviour is not expected to occur when there is low, if any, chance of successful fertilisation (i.e. females should be behaviourally non-receptive). Whilst proestrus and oestrus vaginal smears were grouped together to overcome interpretation issues, it is possible that unsuccessful smears were erroneously categorised as dioestrus when females were actually sexually receptive (proestrus or oestrus). Assaying tarrkawarra receptivity via circulating progestin levels (after Breed & Papps 1976) or inducing oestrus systematically via administration of exogenous gonadotrophins (after Breed & Washington 1991) is recommended to circumvent this short-coming in future studies.

Much recent research suggests that the strength and even the direction of sexual selection changes in constant response to environmental factors, especially in response to changes in population density (Kokko & Rankin 2006; Kasumovic & Andrade 2009; Dreiss et al. 2010). Tarrkawarra population density is known to fluctuate dramatically and it would be valuable to determine whether female mating preferences vary accordingly, ideally by assessing genetic paternity in the field. There is now evidence in captive studies for both large-male selection (via female mate choice) and large-female selection (via fecundity, see Chapter 3). However there are also field data to suggest that social organisation is flexible (Dickman et al. 2010). To explain FSSD in this

species the occurrence of strong sexual selection on small male size must be demonstrated at a critical point in the fluctuating population-density cycle. Alternatively it must be concluded that constraints on male size unrelated to sexual selection account for FSSD in this species.

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Chapter 5

Male mate choice in tarrkawarra (*Notomys alexis*), a mammal with significantly female-biased sexual size dimorphism

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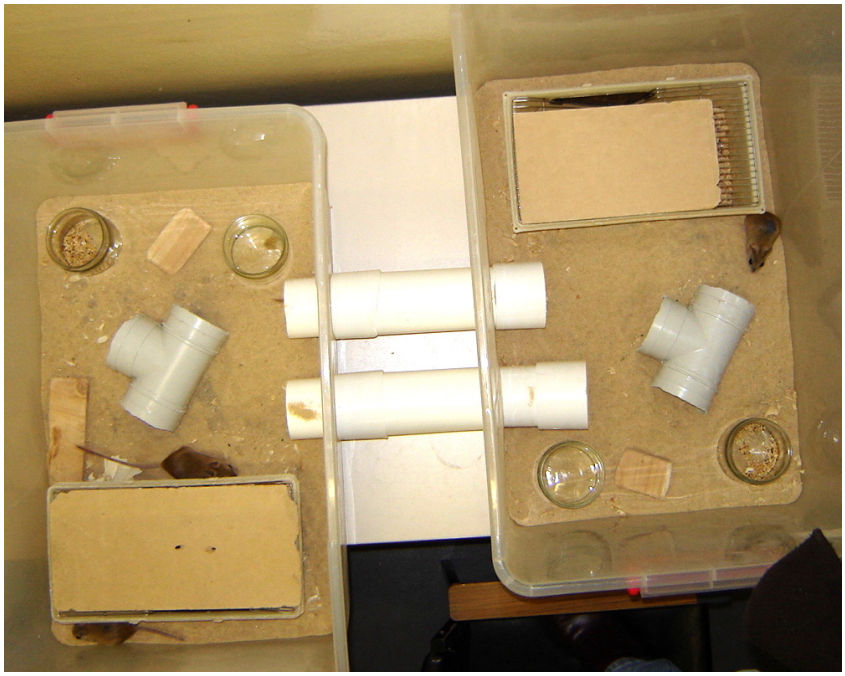
In preparation for submission to Animal Behaviour



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ABSTRACT

Females are typically regarded as the choosier sex but males may also be choosy when the costs of reproduction are high. Mating costs increase, for instance, when there is a reduction of future mating potential, a risk of sperm depletion, extensive paternal care or risk of injury during courtship and copulation. Female aggression will be particularly costly if females are the larger and more aggressive sex, as is the case in tarrkawarra (*Notomys alexis* or spinifex hopping mice). Male tarrkawarra run a risk of injury when encountering an unfamiliar or unwilling mate. Additionally they are sperm limited. To determine if this confluence of mating costs makes male tarrkawarra choosy, I compared male association preferences for opposite sex potential 'mates' with those for same sex 'affiliates'. Although males showed a clear preference for familiar over unfamiliar females and a tendency to prefer large over small females, most barely differentiated between sexually receptive and non-receptive females. However male choosiness based on female quality interacted in complex ways with male sexual experience and size (relative to their same-sex cage mate). The only clear pattern to emerge from these interactions was that small males spent consistently more time with females than did large males, although the effect was not significant. I discuss these results in terms of male self-assessment of quality and argue that small males may compensate for being less preferred in female mate choice scenarios by being more persistent.

INTRODUCTION

Bateman's principle is predicated on reproduction being more energetically costly in one sex than in the other, thus predicting how choosy each sex should be about mates (Bateman 1948). Whilst the principle is not inherently sex-specific, Bateman specifically observed that female costs of reproduction are typically greater than males. By way of example he contrasted the energetically expensive processes of egg production, gestation and lactation with sperm production. Finally he typified males as eager and indiscriminate and females as passive and choosy (Bateman 1948). These stereotyped sex roles have dogged the field ever since (Dewsbury 2005; Bonduriansky 2009; Green & Madjidian 2011). However, there is now an increasing body of research exploring scenarios in which males rather than females are a limited resource. In particular, sperm depletion, female-female competition for preferred mates and male mate-choice are considered as alternatives to conventional forms of sexual selection where these fail to explain the patterns observed (Wedell et al. 2002; Härdling et al. 2008; Bro-Jørgensen 2010).

In mammals, unlike most other taxa, female-biased sexual size dimorphism (FSSD) is rare and poorly accounted for by theory. However sexual selection has largely been neglected as a mechanism for mammalian FSSD because Batesian sex roles are assumed to be particularly entrenched due to the high costs of female reproduction. However, we now know that sperm depletion occurs in preferred male Soay sheep (Preston et al. 2001) and probably also in lekking ungulates (Bro-Jørgensen 2010). The limit that sperm depletion places on female fitness probably also underlies

direct female-female mate competition observed in some of these species (Bro-Jørgensen 2007). However mammalian examples where sperm limitation is also directly linked to male mate choice are elusive. Theoretically when inseminating a large number of females is not possible, and female quality varies, mating preferentially or exclusively with high quality females should maximise the fitness of choosy relative to less discriminating males (Bonduriansky 2001; Barry & Kokko 2010). Essentially this is Bateman's principle but with the typically assumed sex roles reversed.

Male mate choice is intuitively plausible in tarrkawarra (*Notomys alexis* or spinifex hopping mouse). Sperm limitation is well established in this FSSD mammal (see methods) and, although there is little if any male-male aggression, a high level of female-female aggression has been documented (Stanley 1971; Happold 1976). As for most FSSD mammals, the details of the social and mating behaviour of the tarrkawarra are elusive but paternal nest-attendance and pup retrieval has been noted, in captivity at least (W. Breed, pers comm.; pers. obs.). This hints at bi-parental care that would further increase the male cost of reproduction. Further adding to male costs, a recent tarrkawarra study (Chapter 4) indicates a strong direct female mating preference for the larger of simultaneously presented males. In an already sperm limited male, a uniform female mating preference puts preferred males at a high risk of sperm depletion (Wedell et al. 2002) and may select for male mate choosiness.

I tested the hypothesis that tarrkawarra males should exercise mate-choice, and specifically that they should prefer larger over smaller sexually receptive females. Aside from the fact that this species is FSSD, a fecundity advantage to larger female size has been identified (Chapter 3), such that size-biased mating would increase male fitness.

Although females are known to be aggressive towards unfamiliar males, I predicted males would prefer unfamiliar over familiar females as a standard outbreeding strategy.

METHODS

Animals

Apart from being FSSD, tarrkawarra are morphologically similar to the kangaroo rats, gerbils and jerboas of North American, African and Asian deserts (Randall 1994). They are a semi-fossorial nocturnal rodent, endemic to the central arid zone of Australia and, as such, prone to boom and bust population dynamics. Restrictions in male reproductive anatomy preclude intense inter-male sperm competition because, by mammalian standards a) testes:body mass is minimal (Breed & Sarafis 1979; Breed 1981b; Kenagy & Trombulak 1986) b) spermatozoa production is remarkably inefficient (Breed 1981a, 1982; Peirce & Breed 2001), and c) the facility to produce functional vaginal plugs that combat sperm competition is reduced (Breed 1980, 1990). Direct male-male competition is also seldom observed (Happold 1976; Stanley 1971), and the pheromonal cues normally involved in rodent aggression are limited by a diminutive preputial gland (Breed 1981b, 1986). Despite the multiple limitations in their reproductive physiology males can mate successfully with several females a week suggesting that their small sperm reserves nonetheless replenish rapidly (Bauer & Breed 2008). Furthermore, whilst multiple paternity of litters has not been documented (Breed & Adams 1992), copulatory 'locking' does occur (Dewsbury & Hodges 1987), facilitated by enlarged penile spines (Morrissey & Breed 1982) - both adaptations to sperm competition (Eberhard 2009). Likewise, although oestrous cycling enters reproductive quiescence at high or 'peak' field population densities, male spermatogenesis continues unabated

(Breed 1976, 1979, 1992; Bauer & Breed 2008) suggesting that it is not costly to maintain.

Aside from periods of reproductive dormancy at high population densities in the field, tarrkawarra ovulation is spontaneous, and not related to the presence or absence of breeding males (Breed 1975). Full oestrus lasts one to four days (Crichton 1974; pers obs.) and the complete cycle (oestrus, proestrus, dioestrus) lasts six to nine days (Smith et al. 1972; Crichton 1974; Breed & Papps 1976). For the purpose of this study I analysed vaginal smears of female subjects for several weeks before and throughout the experiment and grouped them into two categories: 1) pro-oestrus and oestrus smears, having a preponderance of nucleated epithelial cells and/or cornified cells (both were often present); and 2) dioestrus and indeterminate smears having very few nucleated epithelial or cornified cells and either a preponderance of leucocytes or very few of any cells (after Breed & Papps 1976). Hereafter the first category is referred to as 'sexually receptive' and the second as 'sexually non-receptive'.

Animal Housing

I pair housed twenty-four male and eight female tarrkawarra from a captive bred colony at Macquarie University, Sydney, Australia. Pairings varied in terms of their age and sexual experience but were consistently selected as the smallest and largest of the available same-sex siblings in the colony (Table 1). This maximized the size differences between animals during subsequent testing. Eight of the male pairs were deemed 'focal' males – those which were to 'choose' between potential mates or affliates. The female pairs and remaining male pairs were deemed 'potential mates' and 'affiliates' respectively. Two pairs of maximally unrelated and wholly unfamiliar potential mates

(females) or affiliates (males) were allocated to each pair of focal males. Trials with affiliates were run in an identical manner to those with potential mates and acted as a control, allowing comparison of reproductive (mate) versus social (affiliate) preferences.

Animals were housed at 20-23°C, on a 14:10 h light:dark cycle with lights off at 1400 and fed a millet seed mix supplemented with fresh carrot cubes. Water was available *ad libitum.* Cages were solid-bottom polycarbonate boxes (50 × 40 × 40 cm, L × W × D), furnished with paper pellet substrate, nest boxes and tissue bedding.

Table 1. Age and weight of focal males (those choosing between potential mates or affiliates) by sexual experience

	Focal Males (N = 16)			
	Inexperienced†	Experienced‡	t_{14}	*P*
Mean weight * (g) ± SE	33.86 ± 0.51	33.45 ± 0.38	0.16	0.88
Weight range (g)	25.60 – 42.40	25.40 – 41.50		
Mean age (mth) ± SE	22.12 ± 0.60	24.41 ± 0.95	-2.07	0.57
Age range (mth)	18.83 – 23.91	21.79 – 29.91		

**Mean of weights taken daily to the nearest 0.01g.*
†Virgin males, single-sex housed post weaning.
‡Males housed in mixed-sex groups post-weaning.

The experimental design required that animals presented to focal males in the test apparatus were unfamiliar initially but familiar when presented again in later trials (Table 2). Various studies have suggested that olfactory cues alone are insufficient for rodents to develop individual identification of previously unfamiliar individuals but that this can be enhanced by even very short duration through-mesh physical contact

opportunities (Johnston & Peng 2008). To ensure that both the allocated pairs of potential mates or affiliates could be presented to focal males initially as unfamiliar animals and subsequently again as familiar animals, I used a comprehensive eight-day familiarisation process that has previously been effective with this species (Chapter 4). Firstly, animals to be familiarised were swapped into each other's cages each day shortly before lights went off for the day. Focal males were thereby able to access olfactory cues on female bedding and substrate for one full oestrous cycle. Secondly,

Table 2. Age and weight of potential female mates and male affiliates

	Female potential mates (N = 8)	Male affiliates (N = 8)
Mean weight* (g) ± SE	49.86 ± 0.41	35.13 ± 0.45
Weight range (g)	42.49 – 56.50	26.04 – 41.20
Mean age (month) ± SE	25.13 ± 0.15	23.44 ± 0.27
Age range (month)	21.75 – 26.72	18.85 – 29.92

**Mean of weights taken daily to the nearest 0.01g.*

animals had a degree of physical contact with each other, throughout the eight-day familiarisation period. Contact was made possible by affixing wire mesh grills to the ends of two horizontal lengths of 80mm PVC pipe that joined together the cages of the animals to be familiarised. Animals transited through these pipes, effectively into the neighbouring cage, and made frequent nose and paw contact with the animals there through the grills (1 cm^2 wide gage). Animals also vocalized loudly and persistently to each other through the grills, especially in the first few days of the familiarisation period. The combination of olfactory and physical stimuli over an extended period of

time makes this a very comprehensive familiarisation process (contrast with Zenuto et al. 2007).

Study Design & Procedure

I conducted eight two-choice trials with each focal male making repeated observations of their responding to each potential mate or affiliate. Each potential mate or affiliate was presented to the focal male both before and after familiarisation, as both a smaller and as a larger option and at different stages of the female oestrous cycle~~s~~. Trials were all run within the first three hours of the dark phase of animals lighting cycle. The run order of trials ensured that all familiarity combinations were assessed (Table 3). Additionally, although the difference in relative size of non-focal animals varied from minimal to substantial, only two females were never in a trial against a larger female and only two were never in a trial against a smaller female. At the start of each trial, focal males were placed in the open topped 'hub' of the apparatus (50 × 60 cm, H × diameter; Fig.1) and left for 30 minutes to freely enter, exit and re-enter three lidded arms (100 × 15 × 20 cm, L × W × H). Two non-focal animals were caged into a 10 × 15 × 20 cm (L × W × H) space, behind a wide gauge mesh grill, one at the end of each of the two outer arms. Interaction was possible between animals through the grills. The third, central arm was otherwise the same but unoccupied and controlled for any preferences for enclosed space. Time spent by focal males in this arm was negligible, however, and not included in the analysis. Side placement of non-focal animals in the apparatus was balanced across trials for relative size (compared with the other non-focal animal in the apparatus at that time) and familiarity with the focal male.

On day one of the experimental schedule focal males were observed in the apparatus with the first pair of unfamiliar animals. Home cages were then configured to produce familiarity with the two animals encountered in the apparatus that day. One week later these animals were deemed familiar. For the subsequent 12 days, trials were run every second day presenting focal males with each possible combination of the now familiar animals and a second, unfamiliar, pair of animals. Trial order was randomised. After seven trials the home cages of each pair of focal males were reconfigured to produce familiarity with the second, still unfamiliar pair of non-focal animals. Eight days later these animals were deemed familiar and final trials were run.

Table 3. Familiarity status of non-focal animals on each day they were presented to focal males in the apparatus

		Non-focal animal / potential 'mate'			
Trial	Day	A	B	C	D
1	†1	Unfamiliar	Unfamiliar	-	-
2	8	Familiar	Familiar	-	-
3	10	-	-	Unfamiliar	Unfamiliar
4	12	Familiar	-	Unfamiliar	-
5	14	-	Familiar	-	Unfamiliar
6	16	Familiar	-	-	Unfamiliar
7	‡18	-	Familiar	Unfamiliar	-
8	26	-	-	Familiar	Familiar

†Home cages reconfigured to familiarise focal males with A and B immediately after trial.
‡Home cages reconfigured to familiarise focal males with C and D the following day.

Trials were observed from an adjacent room and filmed from above, with a Sony DV Camera (Model DCR-TRV355E PAL), under infrared lighting for subsequent analysis. Footage was scored by two observers, blind as to the familiarity, relative size and reproductive state of the animals. For each trial the observer scored two observation logs, one for each occupied arm. From these logs I subsequently calculated visit

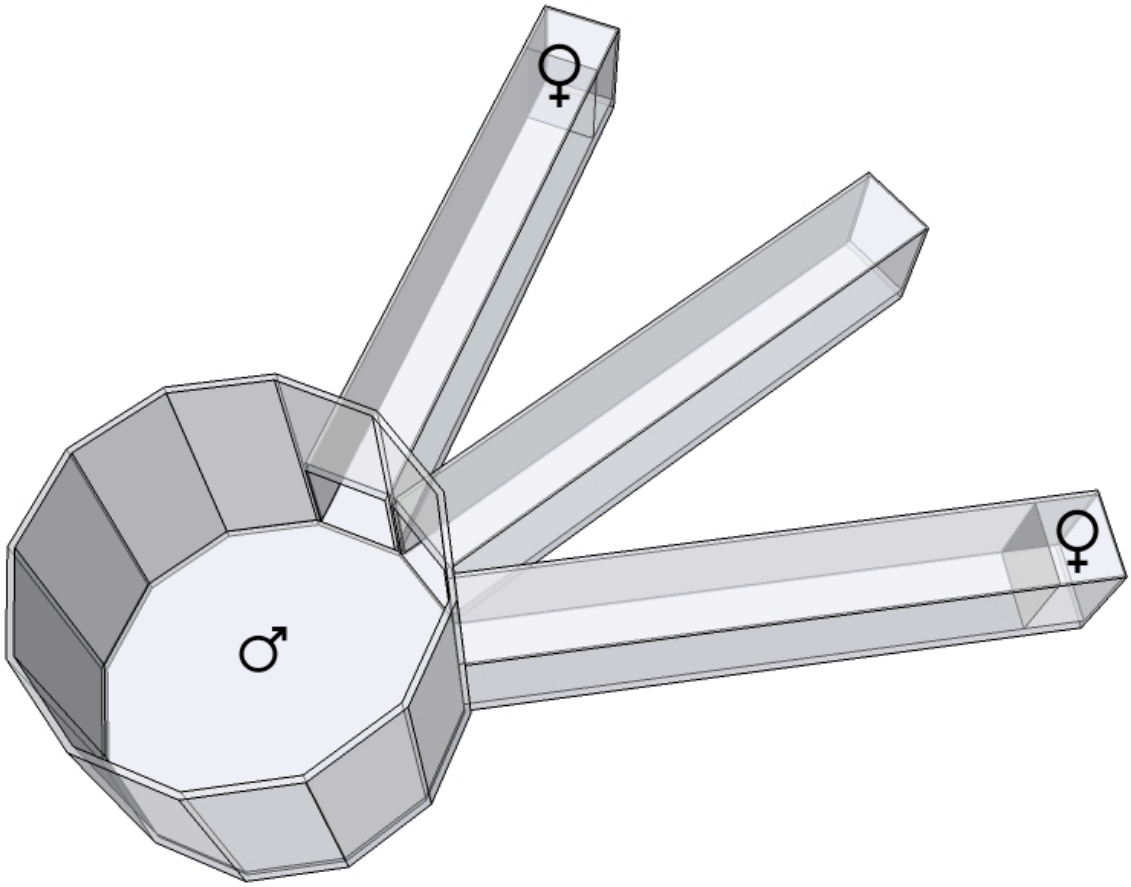

Figure 1. Overhead view of the mate choice apparatus. Three closed-top arms measured 100 × 15 × 20cm (L x W x H) and could be entered from the circular, open-top hub, measuring 50 × 60cm (H x diameter). Wide gauge mesh grills, 10 cm from the closed ends of two outer arms, separated focal from non-focal animals during trials.

frequency and average visit duration of focal males to the space within one body length of the grills. In total there were 256 observation logs from 64 trials with female potential mates and 64 trials with male affiliates. The two blocks of 64 trials were analysed separately using a within and between subjects model.

Statistical Methods

An SPSS mixed linear model procedure was used incorporating three levels: individual males, trials within males, and two observations within each trial. The mixed multilevel model took into account the dependence between trials for the same animal, and responses within trials. Animals and trials made up the random part of the model. This meant that it was not necessary to calculate ratios or difference scores, which are both challenging as dependent variables (Cohen et, 2003). Five fixed factors, each with two levels, made up the fixed part of the model: focal male sexual experience (experienced or inexperienced), focal male size relative to cage mate (smaller or larger), potential mate or affiliate familiarity (familiar or unfamiliar to the male), potential mate or affiliate size (larger or smaller relative to the other non-focal animal in the apparatus on that trial) and potential mate receptivity (receptive or non-receptive, mate trials only). The dependent variable of primary interest was the sum total seconds that focal males spent located within one body length of the grills (‘visit duration’). These data were square root transformed to account for skew. Additionally the number of times males relocated to within one body length of the grills was subsequently analysed.

Ethical Note

The research described in this paper was approved by the Macquarie University Animal Ethics Committee (protocol number: 2005018) and is in accordance with the ASAB Guidelines for the use of Animals in Research.

RESULTS

Altogether sixteen focal males were tested with four non-focal individuals each – either four female 'mates' or four male affiliates. Each non-focal individual was compared at some point with each of the other three, and each was presented both prior to and after familiarisation with the focal animal. Within the first minute of entering the apparatus, focal males had usually entered both occupied arms, and interacted with both non-focal animals through their respective grills. Focal males spent very little time in the unoccupied, centre arm and data from these visits were not analysed.

Focal Male Visiting Duration with Females

The model described in the methods was first applied to the question of whether focal males spent more or less time with females based on female size, familiarity and sexual receptivity and whether this varied according to the male's own size and sexual experience. All but one of the three-way and four-way higher order effects were non-significant ($P > 0.05$). These effects were removed in a stepwise progression from the model followed by the removal of non-significant two-way interactions. The final model retained all main order effects, the two-way effects incorporated in the three-way interaction and one other significant two-way higher order effect (female familiarity by male sexual experience).

There were no main effects of male size, male sexual experience, female receptivity or female size on the total time males allocated to visiting females (Table 4). There was, however a main effect of female familiarity and an interaction of female familiarity with male sexual experience (Table 4). Both sexually experienced and sexually inexperienced males allocated significantly more time to visiting familiar over unfamiliar females but this difference was particularly pronounced for inexperienced males (Table 4; Fig. 2).

The time males spent visiting receptive over non-receptive females varied according to both their own size and their sexual experience (Table 4; Fig. 4(a)). Post-hoc tests of simple effects showed that the effect was driven by large inexperienced males visiting receptive females for significantly less time than non-receptive females ($F_{1,119.1} = 4.98$, $P < 0.05$) and significantly less than small inexperienced males spent visiting receptive females ($F_{1,128.0} = 9.57$, $P < 0.005$).

Focal Male Visit Frequency to Females

Visit frequency (total number of transits to within one body length of grills) has been shown to be a sensitive measure of female mate choice in this species (Chapter 4). To see if males also vary visit frequency in mate choice scenarios, I ran an additional analysis, using the mixed linear model described in the methods, with male visit frequency as the dependent variable. As with the previous analysis, non-significant higher order effects were removed in a stepwise progression leaving a final model that included all main order effects, a three-way interaction and all the two-way effects incorporated in the three-way interaction and two additional significant higher order

effects (female receptivity by female size and female receptivity by male sexual experience).

Table 4. Effects of male sexual experience and size on sum total duration in seconds spent visiting females that differed in familiarity, size and sexual receptivity

Fixed effects*	*F*	*P*
Female familiarity	$35.30_{1,119.12}$	0.00
Female size (relative to alternative female)	$0.362_{1,119.34}$	0.55
Female receptivity	$0.02_{1,120.77}$	0.89
Male sexual experience	$0.07_{1,9.43}$	0.80
Male size (relative to cage mate)	$2.38_{1,126.16}$	0.13
Male size * male sexual experience	$0.00_{1,126.13}$	0.98
Male size * female receptivity	$1.47_{1,120.44}$	0.23
Male sexual experience * female familiarity	$6.38_{1,119.13}$	0.01
Male sexual experience * female receptivity	$0.56_{1,120.55}$	0.46
Male size * male sexual experience * female receptivity	$5.59_{1,120.51}$	0.02

**Data were analysed using a mixed linear analysis and square root transformed to correct for skew.*

There were no main effects of focal male size or male sexual experience, female familiarity or female size on focal male visit rate (Table 5). In contrast with the time allocation analysis however, there was a main effect of female receptivity with males visiting non-receptive females significantly more frequently than receptive females (Table 5; Fig. 3). However, in interaction with male sexual experience, tests of simple effects showed that the preference for non-receptive females was only significant for inexperienced males ($F_{1,94.1} = 10.53$, $P < 0.005$; Fig. 3).

Whilst female size was not significant in the time allocation analysis, it did interact significantly with female receptivity to affect male visit rate (Table 5). Males distinguished between larger females based on female receptivity but they visited the larger non-receptive females significantly more frequently than their receptive counterparts ($F_{1,86.2} = 6.54$, $P < 0.02$) and significantly more frequently than they visited smaller, non-receptive females. When females were non-receptive, males also visited the larger females significantly more than smaller females ($F_{1,94.1} = 10.53$, $P < 0.002$).

Table 5. Effects of male sexual experience and size on visit frequency to females that differed in familiarity, size and sexual receptivity

Fixed effects*	*F*	*P*
Female familiarity	$0.05_{1,96.84}$	0.827
Female size (relative to alternative female)	$3.49_{1,80.49}$	0.065
Female receptive state	$7.99_{1,99.57}$	0.006
Female receptivity * female size	$7.82_{1,86.88}$	0.006
Male sexual experience	$0.17_{1,8.65}$	0.689
Male size (relative to cage mate)	$0.33_{1,63.05}$	0.569
Male size * male sexual experience	$0.01_{1,72.41}$	0.937
Male size * female size	$0.09_{1,61.92}$	0.764
Male sexual experience * female size	$0.01_{1,72.41}$	0.925
Male sexual experience * female receptivity	$3.62_{1,100.71}$	0.060
Male size * male sexual experience * female size	$9.30_{1,63.05}$	0.003

**Data were analysed using a mixed linear analysis (untransformed).*

A greater visit rate to larger over smaller females was also found in association with both male size and male sexual experience (Table 4; Fig. 4(b)). Post-hoc tests of simple effects showed that this effect was driven by the greater visit rates to large,

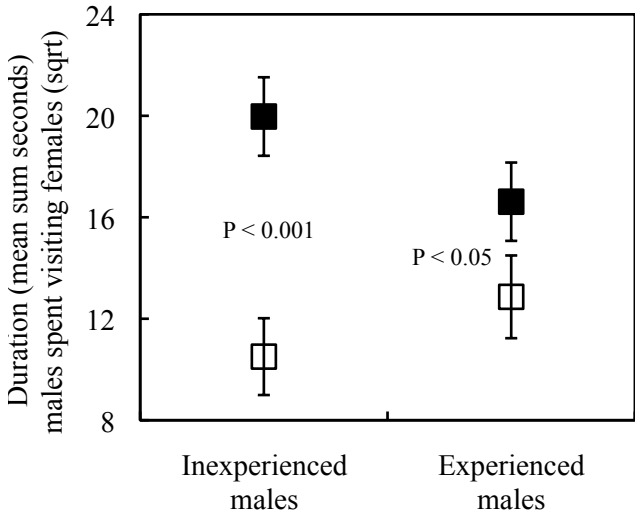

Figure 2. Effects of female familiarity on total time allocated by males to visiting familiar (closed squares) versus unfamiliar (open squares) females. Familiarity is a significant main effect and significant in interaction with male sexual experience. Data points are mean total seconds spent visiting females per 30 minutes observation, square-root transformed, with standard error bars.

compared with small, females by inexperienced males ($F_{1,78.1}$ = 5.50, P < 0.05) and large experienced males ($F_{1,128.0}$ = 9.57, P < 0.005).

Focal Male Visit Frequency to Males

To test whether the effects described above can be confidently attributed to choice between mating alternatives rather than affiliative preferences, data from the observations where focal males were presented with male affiliates that differed in familiarity and relative size were also analysed. The reduced mixed linear models described above were used (dependent variables of visit duration and visit frequency respectively), removing receptivity as a fixed factor and from all interaction terms. Focal males tended to allocate more time to visiting familiar over unfamiliar males ($F_{1,117.5}$ = 2.11, P = 0.15), but no significant effects were found for focal or affiliate male size, sexual experience or interactions of these variables (P value range: 0.21 - 0.72).

Further backward stepwise reductions of non-significant terms in the above analyses did not provide any further explanation of the data.

DISCUSSION

Overall males spent more time associating with familiar than unfamiliar females and this effect was more pronounced in sexually inexperienced than experienced males. Depending on their size and sexual experience most males also tended to be either indiscriminate or spend slightly more time with receptive females than non-receptive females. The notable exception to this was large, sexually inexperienced males who

spent significantly less time with receptive than non-receptive females. In terms of visit frequency, males showed a marginally non-significant tendency to visit larger females more frequently than smaller ones but they did not spend more time with them. Male

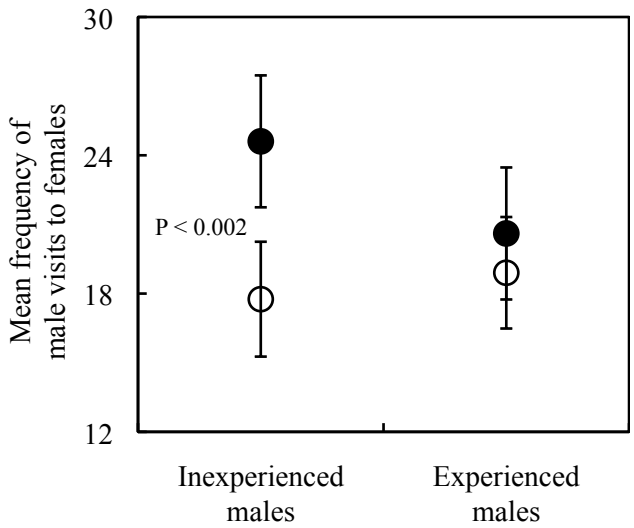

Figure 3. Effects of female receptivity on frequency of male visits to receptive (open circles) versus unreceptive (closed circles) females. Receptivity is a significant main effect and marginally non-significant in interaction with male sexual experience. Data points are mean frequency of visits to females per 30 minutes observation, with standard error bars.

sexual experience also interacted inconsistently with the large-female visit rate preference, such that small males were indiscriminate only if sexually experienced whilst larger males were indiscriminate only if sexually inexperienced. By contrast, the overall effect of focal male size was less ambiguous with small males showing a non-significant overall tendency to spend more time with females than did large males.

The strongest consistent male association preference was for familiar over unfamiliar females. The finding of a familiar-female preference is particularly sound because to be detected it must override typical rodent novelty seeking behaviour (Frynta et al. 2010). Additionally, male tarrkawarra contrasted their familiar-female preference with a tendency towards spending more time with unfamiliar males (P = 0.15), suggesting that novelty seeking overcame associative preferences when opposite sex animals were not available.

One interpretation of the familiar-female preference shown here is that female tarrkawarra are more aggressive towards unfamiliar than familiar males (Happold 1976). Females were able to nose jab, scratch and vocalise through the apparatus grills and may have driven off the attention of unfamiliar males. However, female aggression towards unfamiliar males is not always associated with reduced mating frequency in rodents (Zenuto et al. 2007). Alternatively, strong preferences for familiar partners have been interpreted as indicative of monogamy in a number of rodent species (Shapiro et al. 1986; DeVries & Carter 1999; Ricankova et al. 2007). However, in a similarly constructed female-mate choice experiment (Chapter 4), females showed a strong preference for unfamiliar over familiar males that interacted with female receptivity and the execution of pre-copulatory ‘darting’, indicating an unequivocally mating-motivated

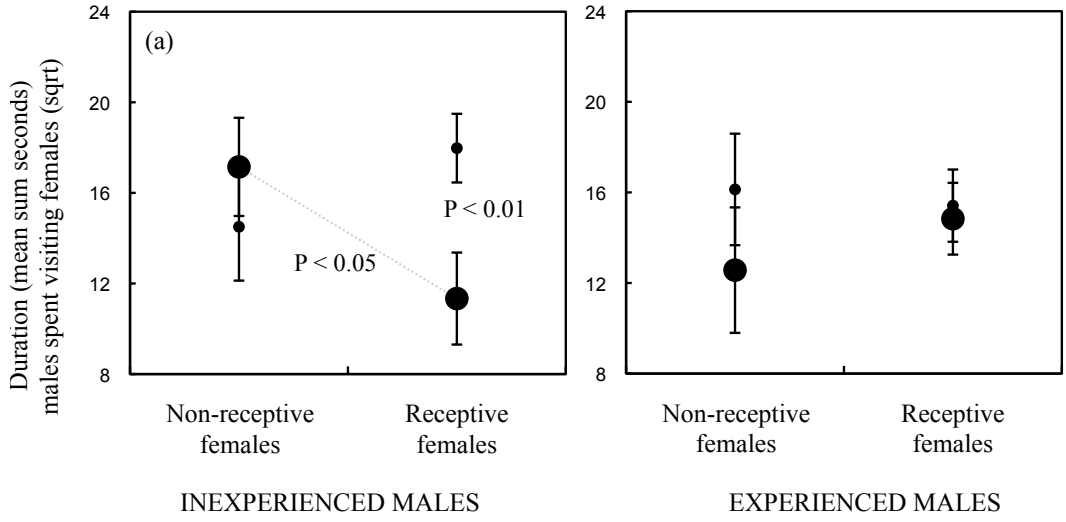

Figure 4. Three-way interaction of male sexual experience and relative home-cage size with (a) female receptivity and (b) female size relative to the alternative in the testing apparatus. Both three-way interactions are highly significant. Data points are mean female association indices for the smaller (small circles) and larger (large circles) males of the home-cage pair, as measured by (a) total time spent with females and (b) total visits made to females per 30 minute observation. Duration spent visiting females is square root transformed to account for skew. Bars indicate standard errors.

preference. Attributing the male behaviour observed here to mate choice is more problematic.

One explanation for a male preference for familiar over unfamiliar females is that unfamiliar females may be resistant to mating prior to familiarisation (Patris & Baudoin 1998). However, tarrkawarra females have a clear preference for unfamiliar males and direct mating initiation behaviours towards them (Chapter 4). Alternatively a preference for familiar females may reflect a persistence or 'bird in the hand' male strategy. Maintaining close proximity with a local, familiar female that will shortly be in oestrus (or post-partum oestrus), may ensure reproductive success more effectively than delaying mating further with lengthy mate searching, risky approach, familiarisation and courting. This is a similar strategy to that adopted by males of the FSSD spotted hyena (Szykman et al. 2001). Under these ecological scenarios familiar males might have an advantage despite a female preference for unfamiliar males (Frynta et al. 2010).

Male preference for larger over smaller females is not a surprising finding and is in line with male mating preferences observed in many other animals (e.g. humpback whales Pack et al. 2009; Soay sheep Preston et al. 2005; see Bonduriansky 2001 for a review). Because size and condition, and condition and fecundity, are highly correlated, quality-based mate discrimination is likely to reflect this (Blanckenhorn 2000). Additionally the age / fecundity function is parabolic (maximum fecundity in middle age) for many females and smaller females may be judged less attractive if their size suggests adolescence (Jones et al. 2008). In tarrkawarra, growth continues well into late adulthood (Chapter 2) and fertility peaks in middle age (Breed 1979; Telfer & Breed

1976). However, the females in this experiment were all fully-grown and age matched. In fact it is surprising that the large-female preference recorded here was not greater. Whilst it was a strong main effect, it was only expressed in response to non-receptive females, which is counter-intuitive in terms of mate choice. Nonetheless, even a low level of direct male mating preference for larger females could contribute cumulatively to the maintenance of FSSD via selection on female size (Bonduriansky 2001).

As with female size, the response of male tarrkawarra to female state of receptivity was not clear-cut. A main effect suggested a preference for non-receptive females but experienced males were indiscriminate based on female receptivity and on par with inexperienced males in their responses to receptive females. Males may not be strictly ambivalent to female receptive state, however they were inconsistent in their responses, dependent on their own sexual experience and size.

Self-assessment of quality is one means by which differences in responses may become associated with the respondents' phenotype. Considerations of self-assessment are relatively new in the field of mate choice but recent studies suggest that choosiness may be lessened in animals that are given quality handicaps (Burley & Foster 2006; Holveck & Riebel 2010; Griggio & Hoi 2010; Royle & Pike 2010). For example, poorer quality zebra finch and house sparrow females (those with clipped wing feathers and those of lighter weight respectively), both associated preferentially with less attractive males whilst higher quality females were indiscriminate (Burley & Foster 2006; Griggio & Hoi 2010). Other studies have shown that males adjust their courtship displays according to female responses and suggest that social feedback may be the mechanism by which self-assessment, and self-regulation of behaviour, is achieved (Patricelli et al.

2002; Royle & Pike 2010). Self-assessment of phenotype may also explain why smaller males in this study spent more time with females than larger males did. Female tarrkawarra prefer larger males (Chapter 4) and the small-male effect in this study may indicate a compensatory response to negate that preference.

The mixed effects of male sexual experience in this study may also, or alternatively, reflect self-assessment of quality. Variance in unspecified phenotypic traits may have differently affected the previous female encounters of sexually experienced males (prior to this experiment), with rejection expected to reduce their choosiness (Fawcett & Bleay 2009). Male tarrkawarra in this study may have also or alternatively received social feedback from their same-sex cage-mates. For instance, in many species male dominance is positively correlated with size and smaller males are subject to social defeat effects of subordination (Andersson 1994, but see Schulte-Hostedde & Millar 2002 for an exception). Dominant animals are often more reproductively successful and subordinates may have to be relatively less choosy or more persistent to achieve matings (Kokko 2005). Male tarrkawarra are, however, remarkably amicable in their intra-sexual interactions and are noted for demonstrating only very rare instances of low levels male-male aggression (Happold 1976). On this basis a strong dominance hierarchy seems unlikely. Nonetheless, the main effect of focal male home-cage size (smaller or larger than same-sex cage affiliate) is suggestive of either a self-assessment or phenotypic size-based behavioural difference.

Alternatively the tendency of small males to be more attendant on females in this study may reflect different social experiences to those of larger males during ontogeny. For instance litter size and pup weight are inversely correlated and the heavier on

average individuals that are produced in small litters also have more aggressive and competitive behavioural styles (Rödel & von Holst, 2009). Behavioural differences can be shaped by early experiences of sibling competition and differential maternal investment and be persistent and ongoing effects (Bautista et al. 2009).

One of the strengths of the design used here was the repeated familiarity measure within and between non-focal animals. This controlled for variation in individual female phenotype and increased confidence that the male preference for familiar over unfamiliar females is robust. A similar manipulation of male sexual experience may have provided more conclusive results. However, it is important to also be aware of the artificial nature of the two-choice mate preference scenario. Females are likely to be encountered sequentially rather than consecutively in the field, especially during times of low population density (Wagner, 1998). Mate scarcity should reduce male choosiness across all conditions. Also males, and particularly inexperienced males, may rely on more than just the olfactory cues of receptivity to trigger mating-motivated behaviour. For example, in rats, olfactory cues are not a necessary condition to elicit the pre-copulatory sequence of male mating behaviours (chasing, genital investigation, mounting). Females can still elicit chase and copulatory sequences from males deprived of olfaction using proprioceptive cues such as nose pokes and flank contact. These physical cues are particularly important for sexually inexperienced male rats (Barnett 1963; Beach 1940, 1976). Whilst limited contact was possible between males and females in this experiment it may have been insufficient for female execution of solicitous behaviours such as darting (Chapter 4).

Despite the limitations of this study, it is apparent that male mate choice in tarrkawarra involves a complex interplay of external cues as to female quality with male self-assessment or behavioural styles based on size and / or feedback from previous social and sexual encounters. However, a high level of individual and context-dependent variation in male mate preference is not out of line with current theory (Fawcett 2003). Notwithstanding individual male variation, a male preference for large females seems likely to contribute to selection on large females in this species. Likewise small-male compensatory courtship behaviour may counterbalance the female preference for large males and result at least in stabilising selection on male size (Blanckenhorn 2005). Interestingly the field data suggest that mean male weights are lower at times of low, compared with high, population density (Bauer & Breed 2008; Breed 1979, 1992). This suggests that smaller males may be more viable and/or more reproductively successful during these genetic bottleneck events. Wide female spatial distribution is characteristic of these events and the greater investment small males make in mate acquisition and attendance may therefore increase their fitness over that of large males. Ultimately field studies of genetic paternity are needed to determine the veracity of this hypothesis but it seems likely that FSSD in this species is driven via selection on both male and female size.

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CONCLUSIONS

In this thesis I sought to demonstrate the value of tarrkawarra as a model species with which to further our understanding of mammalian female-biased sexual size dimorphism (FSSD), particularly in the context of sexual selection via mate choice. In surveying other potential model mammalian FSSD systems (Chapter 1) it became clear that there is currently a dire need for data on the life histories of many more species on which to construct hypotheses to explain mammalian FSSD. Tarrkawarra is a particularly promising species with which to start because intra-specific variation is indicated in some key traits that are thought to contribute to the evolution and maintenance of SSD. Namely these are sexual selection, spatial distribution of conspecifics (or population density) and the ontogeny of body size itself. The first of these is addressed predominantly in Chapters 4 and 5, the latter two in Chapters 2 and 3.

Population density and FSSD

To address my broad aims, I first synthesised the published data on tarrkawarra body mass at the extremes of the range of population densities that naturally occur (Chapter 2) (Bauer & Breed 2008; Breed 1979, 1992; Dickman et al. 1995; Dickman et al. 1999; Predavec 1994). I identified a pattern in these data whereby FSSD is a consistent feature of low but not high-density populations. Specifically mean high-density male weights are elevated and near parity with females, suggesting that male size is under positive selection in these conditions. This follows logically from theory that relates increased spatial incidence of mating opportunities to an increase in the optimal level of polygyny (Andersson 1994) and, in turn, increases in the degree of polygyny to increased strength

of selection on large male size (Lindenfors et al. 2007). This suite of theories is founded on quantified examples from multiple different species that each vary on one or all of these factors. In a few cases, however, environmental variation is sufficient between populations of the same species to generate similar effects at the intra-specific level. The African striped mouse (*Rhabdomys pumilio*) is one such case (Schradin & Pillay 2005) and recent field observations of social flexibility in tarrkawarra (Dickman et al. 2010) suggest that this species may be another. Scramble competition is presumably the default low-density tarrkawarra social organisation given the associated forage scarcity at these times. There are numerous reasons why selection might favour small males under scramble competition (Blanckenhorn 2000). Conversely, at high population densities I predict that the optimal mating system changes to one in which selection on small male size is either relaxed or reversed.

If the FSSD / population density interaction, observed for tarrkawarra in the field, could be replicated in captivity and systematically manipulated, the results could potentially be most illuminating. I attempted to do just this (Chapter 2). However, high-density captive housing did not enhance male size as expected. Instead the manipulation retarded the growth of both sexes, and especially females, producing sexual size parity relative to a low-density condition. Whilst large by captive standards, 11m^2 enclosures combined with the social cues provided by more or less cage mates, was insufficient to replicate the levels of FSSD observed at high and low-density field conditions. Although tarrkawarra are small (25-35 grams in the field) they can travel up to 14 linear kilometres in a fortnight (Dickman et al. 1995). Additional cues indicating wide spatial distribution of mates (such as the removal of *ad libitum* food and water or increased effort required to attain food and water) may be needed to simulate field conditions in

captive studies. Alternatively (or additionally), future studies should either use field-trapped adults or conduct field-based experiments at different population densities. Either scenario is a long-term undertaking as droughts tend to be widespread and periods between high-density population events sometimes last for many years.

The ontogeny of FSSD

Tarrkawarra size will necessarily be a function of rearing factors as well as population density. This is because FSSD is present in litters of neonates (Chapter 2) creating sex-differentiated energetic requirements of offspring throughout the period of maternal investment. In particular the number and sex of siblings will affect individual pup growth (Chapter 3). In tarrkawarra, as in other species, maternal investment is expected to be sensitive to tradeoffs between number and size of sons versus daughters. In support of this female, but not male, pups gain more weight in small litters and are less likely to be reared in large litters. Female offspring, again unlike males, also gain more weight when reared in litters biased towards their own sex. These litter size and sex-ratio effects suggest a level of sex-differentiated maternal investment which is typically assumed to reflect different fitness potential of the sexes (Trivers & Willard 1973). Two ways in which female fitness may be increased by larger size are identified in this thesis. Firstly larger females produced more pups per litter (Chapter 3) and, secondly, larger females were preferred over smaller females in a male mate choice scenario (Chapter 5).

Female-biased maternal investment is intuitively adaptive for FSSD species in the same way that male-biased investment is intuitively adaptive (and common) for male-biased SSD species. The sex for which fitness depends on weight gain to sexual

maturity (and beyond) should receive a 'silver spoon'. There is now evidence of this sex role reversal in two FSSD mammals that I know of - tarrkawarra (Chapter 3) and the bank vole (Bondrup-Nielsen & Ims 1990; Koskela et al. 2009). It will be interesting to see if this is a consistent pattern in FSSD mammals and whether it varies with the degree of FSSD in species where this is flexible (as it is in both tarrkawarra and bank voles). Sex-differentiated maternal investment is one mechanism by which the degree of FSSD in the field may be adjusted. I predict that conditions associated with low tarrkawarra population density will cue maternal investment in larger daughters, whilst conditions at high population densities will cue investment in larger sons. Mixed effects are expected in captivity unless low vs. high population field conditions can be adequately simulated.

Sex ratio adjustment is a one form of maternal investment that should be further explored in tarrkawarra given the gestation effects observed in Chapter 2. A number of well established conditions produce sex-differentiated effects on fetus mortality, viability and post-natal growth (Badyaev 2002) and these may also facilitate a degree of mammalian control over litter sex-ratio (Hardy 1997). Delayed fetal implantation (diapause) can prolong gestation up to 16 days beyond the 32-day minimum in tarrkawarra (Breed 1979) and correlational data now suggest that lengthy gestations are associated with male-biased litter sex ratios (Chapter 3). Sex-differentiated mammalian implantation rates occur in association with variation in photoperiod, maternal nutrition, condition and nursing status (Renfree & Shaw 2000) as well as circulating testosterone and glucose levels (e.g. Cameron & Linklater 2007; Gutiérrez-Adán et al. 2001; Helle et al. 2008) around the time of conception. Environmental regulation of sex-differentiated diapause via any one or a combination of these mechanisms may adjust tarrkawarra

litter sex ratios in ways that maximise fitness given the prevailing environmental conditions, population density and mating system.

Sexual selection and mating system in FSSD

Female preferences for small mates complement large-female fecundity selection in non-mammalian FSSD species (mostly arachnids). Similar preferences have not been documented in FSSD mammals but this may simple reflect study biases that preclude sexual selection research on these species. However, fecundity selection on large female size (Chapters 2 and 3) is not sufficient to explain mammalian FSSD (see Chapter 1 for rationale) and selection on small male size (or, at least, relaxed selection) must also occur (Lindenfors et al. 2007). I therefore investigated whether female tarrkawarra show a small-male preference in a mate choice scenario. However female mate choice clearly favours larger over smaller males (Chapter 4). Enhanced selection on genetically larger male size may also occur via a 'differential allocation' type effect whereby tarrkawarra females invest preferentially in male offspring when mated to attractive males (Harris & Uller 2009). Support for this comes from the finding that litters born to larger males also had more male-biased sex-ratios (Chapter 3).

Female preferences for large mates are problematic for explaining FSSD unless small or otherwise less-preferred males can somehow circumvent female preferences and achieve greater mating success despite them. Poeciliid females also show a large-male mating preference that small males surmount by increasing their gonopodial thrusting efforts relative to large males when females are non-receptive (e.g. Bisazza & Pilastro 1997). Likewise there is some evidence that smaller tarrkawarra males may be more persistent in courtship and / or mate attendance than large males. Males that were

designated ‘small’ relative to their same-sex cage mate demonstrated greater female attendance levels in a mate choice scenario than those designated as ‘large’ (Chapter 5).

There are at least two candidate mechanisms for male size–differentiated responding to females. The first is individual variation in behavioural profiles. This posits that, just as early body size scales to adult size (Chapter 2; Rödel et al. 2008), different early experiences (intrauterine position, maternal investment or relative success in sibling competition, for instance) can correlate with adult behavioural repertoires, attractiveness and ultimately fitness (Clark et al. 1992; Rödel & von Holst 2009). Components of tarrkawarra ontogeny may likewise produce size-differentiated adult males responses, including responses to females, depending on whether males were smaller or larger pups. Observational data on mother-offspring and sibling interactions is required to address this hypothesis and would also add to the data on maternal investment in this species (Chapter 3).

The second candidate mechanism for male size–differentiated attendance to females is a response to self-assessment of phenotype. This effect has recently been shown in zebra finch where handicapping male attractiveness increased the courtship effort they directed at poor versus high quality females (Royle & Pike 2010). Testing for male self-assessment of relative size in tarrkawarra should assess relative female attendance of similarly sized males housed with either a much smaller or a much larger male. Differences in behaviour can then be attributed to male self-assessment of relative quality rather than to absolute size. Unfortunately these comparisons were not possible in the study presented here because I deliberately selected male cage mate pairs so that small and large weight distributions overlapped minimally.

As well as individual variation, population level variation in social organisation and mating systems seems increasingly likely in tarrkawarra (Bradley 2009; Dickman et al. 2010). Attempts to define the mating system as either monogamous or promiscuous will likely continue to fail to account for all the contradictory behavioural and reproductive anatomical observations. Opposite effects of familiarity on female and male mate choice demonstrate this point. Males had a clear preference for familiar females (Chapter 5) but females clearly preferred unfamiliar males (Chapter 4). The former pattern is typically associated with monogamous species and the latter with polygynous (e.g. Salo & Dewsbury 1995; Ricankova et al. 2007). However the same dichotomy can be found in a single species for a myriad of reasons including whether or not an individual's mother was concurrently nursing and gestating (Clark et al. 2006).

Greater male attendance to familiar over unfamiliar females despite female preference for unfamiliar males may reduce inter-sexual aggression. Males may establish female associations prior to oestrous events, maintain close proximity and 'wait it out'. This is akin to the strategy employed by male hyena, arguably the best studied of the FSSD mammals (Szykman et al. 2001). However, ultimately the only effective measure of social organisation in any given tarrkawarra population will be to assess individual paternity rates in the field. This is one of the more promising future areas for field work on this species as it is less logistically fraught than behavioural studies on a burrowing animal. Trapping protocols and sites are already well established (Dickman et al. 2010). The genetic markers are still to be identified for tarrkawarra, however the technology to do this is increasingly available and affordable.

As a final note, data on size-differentiated tarrkawarra male performance at tasks other than mate choice are also needed. For instance, differences in rate of energy metabolism, basal (BMR) and maximum aerobic metabolic rates are all selective agents on male size that can affect fitness (Boratyński et al. 2010). Data that were not included in my thesis suggest sex-differentiated metabolic and activity budgets in that male tarrkawarra consume a significantly greater proportion of their body weight per 24-hour period than do females. If these traits also vary within-sex the nature of these differences will add greatly to our understanding of FSSD in this species.

Summary

In summary, whilst there is still much to determine about the basis and maintenance of FSSD in tarrkawarra, the apparent social flexibility of the species makes it an ideal system with which to test and challenge conventional understandings of mammalian SSD. It's ontogenetic malleability should be harnessed to help us understand the conditions under which FSSD is and is not selected for without having to make inter-species comparisons (see Chapter 1 for a discussion of the advantages of studies of intra-species variation).

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