

**Plasticity of Maternal Care and Seasonal Manipulation of Masculinity
in *Peromyscus maniculatus***

by

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ABSTRACT

PLASTICITY OF MATERNAL CARE AND SEASONAL MANIPULATION OF MASCULINITY IN *PEROMYSCUS MANICULATUS*

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Individual variation in maternal care has been shown to have important consequences for offspring development, physiology, and behaviour. However, investigating the evolutionary consequences of differences in maternal behaviour requires the extension of well established protocols for measuring maternal behaviour in the lab to a wild rodent species. I investigated maternal care, and associations between secondary sex ratio and individual masculinity in regards to local resource competition, in a wild rodent: the woodland deer mouse (*Peromyscus maniculatus gracillis*). I demonstrated that 1) wild mice display similar behaviours as laboratory mice; 2) maternal care is a plastic phenotype that decreases as population density increases over one breeding season; 3) sex ratio is not manipulated in response to local resource competition; and 4) sex ratio and masculinity are correlated in this system, and decrease with increasing population density. I conclude that maternal care and offspring masculinity change over the course of one breeding season in a wild rodent, *Peromyscus maniculatus*.

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CHAPTER I

GENERAL INTRODUCTION

When nurturing her offspring a mother transmits information about her current environment to the subsequent generation (Mousseau and Fox 1998). This form of preparing offspring for the conditions they will encounter as adults is termed a maternal effect (Bernardo 1996), and in mammals, maternal effects are especially relevant due to their long period of offspring dependence after birth (Clutton-Brock 1991; Reinhold 2002). Two ways that mothers may prepare offspring for their environment is through postpartum maternal care (Champagne et al. 2003a; Champagne 2009) and sex ratio manipulation (Monclùs and Blumstein 2012). Here I define maternal behaviour to be the behaviour a mother displays in the vicinity of her offspring for the first week postpartum, which to date, has primarily been studied in non-human primates (Suomi 1997), domestic animals (Jensen 1986), and those raised in a laboratory (Myers et al. 1989a; Champagne et al. 2003a; Champagne et al. 2007). This thesis seeks to understand maternal care, as well as variation in offspring sex ratio and individual masculinity in a wild rodent, the woodland deer mouse (*Peromyscus maniculatus gracillis*).

MATERNAL CARE IN RODENTS

Laboratory research has shown that female rodents consistently differ in their maternal behaviour and that dams display the same behaviour between litters (Champagne et al.

2003a; 2003b; Weaver et al. 2004). Further, the quantity of nursing or licking/grooming behaviour that an individual receives within the first few days of life regulates adult behaviours such as fearfulness, anxiety, response to stress, and mothering style (Caldji et al. 1998; Champagne et al. 2003a, Champagne 2009). This epigenetic regulation is determined through the density of hormone receptors in the infant brain such as estrogen, glucocorticoids, and oxytocin. Laboratory dams that frequently demonstrate nursing and licking/grooming behaviours produce pups with increased hormone receptors within their brain (Liu et al. 1997; Liu et al. 2000; Champagne 2009). Alternatively, offspring who receive reduced maternal behaviour (*i.e.*, infrequent nursing and licking/grooming behaviours) have reduced estrogen, glucocorticoid, and oxytocin receptors within their brain, which results in a heightened stress response, anxious behaviour, and a decrease in maternal care (Caldji et al. 1998; Francis et al. 2002; Meaney 2001).

These variations in maternal behaviour have been eloquently outlined in numerous laboratory studies. Connections between maternal care and offspring stress response (Caldji et al. 2000; Francis et al. 2002), physiological changes such as alterations in blood pressure (Myers et al. 1989a), and endocrine functions through alterations to the density of hormone receptors in targeted tissues as well as number of dendrites within the brain (Weaver et al. 2004; Saplosky 2004; Hasselt et al. 2012; Labonte et al. 2012) have been made where laboratory rats (*Rattus*) or mice (*Mus*) are the animal models used. Despite understanding the physiological mechanisms behind these associations, we do not currently understand whether these associations influence offspring phenotype under natural conditions and whether these associations can change in a naturally fluctuating environment.

Although the epigenetic association between maternal care and offspring phenotype in wild animals is undetermined, the advantage of receiving either high or low levels of maternal care may vary. That is, whether an individual receives frequent, or infrequent, maternal care may be beneficial or detrimental depending on the environmental conditions the individual experiences as an adult. According to laboratory studies, neuronal changes such as these are sustained into adulthood and affect an individual's behaviour throughout their lifetime, however, in order to test ultimate hypotheses regarding maternal care we require a wild study system.

SEX RATIO MANIPULATION AND OFFSPRING MASCULINITY

Fisher (1930) originally predicted that frequency-dependent selection should result in a balanced sex ratio. However, we repeatedly see skews in sex ratio in wild populations. Four non-mutually exclusive hypotheses have been proposed to explain why mothers should differentially invest in their offspring based on sex. These include Trivers-Willard (maternal body condition; Trivers and Willard 1973), Local Resource Competition (mating system and population density; Clark 1978; Silk 1983), First Cohort Advantage (body size and rate of reproductive maturation; Wright et al. 1995), and Extrinsic Modification (environmental variables; Post et al. 1999; Forchhammer 2000).

One of the most commonly tested theories of sex ratio manipulation is local resource competition (LRC). This theory applies to species that experience extreme variation in population density and exhibit sex specific dispersal (Clark 1978; Silk 1983; Sikes 2007). In periods of high population density selection should favour females that

produce the dispersing sex in order to decrease competition for local resources within the natal territory (Clark 1978). This concept has been well studied in a variety of taxa, but when applied to *Peromyscus*, who exhibit male-biased dispersal, we would expect that more males ought to be produced during periods of high population density. However, within most species, there is individual variation in dispersal distance within a sex; not all males will disperse and not all females are philopatric (Cantoni et al. 1999; Nunes et al. 1999).

The sex ratio of a litter may profoundly impact parental fitness because males and females often differ in their reproductive output. Sons and daughters can produce different numbers of grand-offspring, where in most instances, a son's fitness is correlated with individual quality whereas females almost always reproduce (Monclùs and Blumstein 2012). Therefore, sex ratios may vary with the costs and benefits of producing high quality male versus female offspring (Clutton-Brock and Iason 1986). Parents may alter the number of sons and daughters they produce within their lifetime through physiological mechanisms (*i.e.*, primary sex ratio), or the quality of those offspring through behavioural mechanisms (*i.e.*, secondary sex ratios), but the ultimate goal is to produce as many grand-offspring as possible (Trivers & Willard 1973; Armitage 1987; Silk & Brown 2008; Monclùs and Blumstein 2012).

In addition to parental fitness, the presence of siblings, and the sex of those siblings, may have long lasting influences on an individual's phenotype and fitness (Monclùs and Blumstein 2012). Several laboratory studies have demonstrated that sex specific behaviour and reproductive success may be associated with male-biased sex ratios (Clutton-Brock and Iason 1986; Ryan and Vandenberg 2002; Monclùs and

Blumstein 2012). For example, the number and position of males *in utero* increases prenatal testosterone exposure to other litter-mates (Cantoni et al. 1999; Ryan and Vandenberg 2002; Lummaa et al. 2007). This change in pre-natal environment appears to influence female lifetime reproductive success by causing adult females to display masculine mating behaviours and be less receptive to mating with males (Rines and vom Saal 1984; Quadagno et al. 1987; Ryan and Vandenberg 2002; Monclùs and Blumstein 2012). Masculinity can be indirectly measured by an individual's ano-genital distance (AGD); the length of the peritoneal tissue between anus and genital papilla. In rodents, AGD increases with prenatal testosterone exposure and differs between males and females, where males have larger AGDs than females (Ryan and Vandenberg 2002; Monclùs and Blumstein 2012).

The connection between sex ratio and masculinity of a litter has been well established; there is a positive correlation between the percent of the litter that is male and the masculinity of sisters (Ryan and Vandenberg 2002). However, current sex ratio theory does not take the masculinity of offspring into account. Litter sex ratio may be altered by producing either male or female offspring, which will affect parental fitness, but perhaps the masculinity of offspring represents a fine scale alteration to sexually dimorphic phenotypes in offspring. Masculinity and litter sex ratio can be altered by the prenatal environment (Krackow 1990; 1995; 1997; Ryan and Vandenberg 2002; Cameron 2004; Rosenfeld and Roberts 2004; Kemme et al. 2006; Love et al. 2005), but changes to the postnatal environment, such as variations in maternal care, may also have a profound influence on individual masculinity (Moore and Morelli 1979; Moore 1992).

WOODLAND DEER MICE

Woodland deer mice (*Peromyscus maniculatus*) are small (18 to 30g) nocturnal mammals that live in mature deciduous forests across North America (Svihla 1932; Hamilton 1941; Wolff and Hurlbutt 1982; Kirkland and Layne 1989). They are good subjects for both field and laboratory investigations due to their abundance, ease of capture, handling, and marking, and their adaptability to captivity (Kirkland and Layne 1989; Wolff 2003).

Many traits of *P. maniculatus* vary depending on geographic location (Millar 1984), masting of primary food sources (*i.e.* maple, oak, or beech trees), and the resulting within and between year fluctuations in population density (Hamilton 1941; Falls et al. 2007). However, in central Ontario, the average breeding season extends from late April through to early September (King 1963). In the subspecies *P. maniculatus gracilis*, females regularly become reproductively mature by 50 days of age and will remain in oestrus for several hours (Kirkland and Layne 1989). One exception occurs with females who are born towards the end of the breeding season. These females over-winter and go into their first oestrus the following spring, rather than mating in the fall (Millar 1994; Kirkland and Layne 1989). The gestation length of *P. maniculatus gracilis* varies depending on lactation status, but is approximately 24 days (Svihla 1932; Kirkland and Layne 1989). Immediately after parturition, most female deer mice enter a postpartum oestrus for several hours, during which time she may once again conceive. A female may produce between one and four litters during the season, and each litter may contain between one and eight offspring (King 1958; Wolff et al. 1985). Mothers nurse their litters for approximately 24 days (Millar 1984); there is no paternal care in deer mice. Litters begin to emerge from their natal nest between 22 and 37 days of age, after which

they no longer receive any maternal care (Svihla 1932; King 1963). By this point the female is no longer lactating and if she is not already pregnant (*i.e.* did not conceive during her postpartum oestrus) she will once again go into oestrus.

Studies involving maternal investment in *Peromyscus* are numerous (Rheingold 1963; Kirkland and Layne 1989). However, little is known about maternal behaviour in this genus, and studies regarding sex ratio manipulation have reported varying results. In terms of maternal behaviour, it is known that a mother will commence her maternal behaviour directly after birth by first licking her pups. It appears that *P. m. gracilis* has the highest nest attendance of any subspecies (Rheingold 1963). However, the exact maternal behaviours occurring in the nest have not yet been explored. Because of this lack of information, it is unknown whether the same findings regarding maternal behaviour in laboratory rodents apply to wild *P. maniculatus*. In terms of sex ratio manipulation, there is little information as to primary (at conception) sex ratios or individual masculinity, however, observations from previous studies have suggested that secondary (at birth) and tertiary (at first capture) sex ratios may vary with litter size, a mother's physiological condition or Julian date, (Goundie and Vessey 1986; Kent 1992; Zuleta and Bilenca 1992), precipitation or temperature (Havelka and Millar 1997), population density, (Canham 1970; Stirling 1971; Kaufman and Kaufman 1982; Myers et al. 1985; Kaufman and Kaufman 1987; Shibata and Kawamitchi 2009), or the stability of the social environment (Kemme et al. 2006). We know that the sex ratio, the probability of dispersal, and most likely masculinity varies in *Peromyscus*. However, we do not have a clear idea of the fitness consequences for these observed variations.

RESEARCH GOALS

My primary research goal was to quantify maternal care in a wild rodent and to compare the variation and plasticity of these behaviours to previous research on laboratory rodents. My secondary goal was to investigate whether individual masculinity is associated with sex ratio manipulation in the context of local resource competition.

To quantify maternal care in *Peromyscus maniculatus gracillis* I observed maternal behaviour, maternal provisioning, and maternal responsivity in wild individuals. Pregnant female deer mice were brought into a field laboratory setting, allowed to give birth, and then released in hopes that they would be caught while pregnant with their next litter. If maternal care in wild *Peromyscus* is influenced by the same mechanisms as laboratory individuals, then I predicted that:

- 1) maternal care would be comparable to maternal care in laboratory individuals, where the proportion of time spent nursing varies within one night, but decreases across the first six postnatal days.
- 2) dams should consistently differ in the frequency of maternal behaviours regardless of natural changes in the environment throughout the breeding season.

I tested the sex ratio theory of local resource competition on a 16-year long-term study of *Peromyscus maniculatus* in Algonquin Provincial Park. I determined the relationships between sex ratio and population density across each breeding season, in the spring (May), and in the fall (August). I also used ano-genital distance and population

density data from the 2011 breeding season involving maternal care (see Chapter II) to test the association between litter sex ratio, population density, and ano-genital distance in the context of local resource competition. Local resource competition predicts that dams should vary their sex ratio in relation to population density in order to increase the probability of their offspring dispersing during periods of high population density. However, when adding the concept of variation in masculinity to this theory then I hypothesize that alterations to offspring masculinity should have similar fitness consequences as alterations to offspring sex ratio. I predicted that:

- 1) dams should vary the masculinity of their offspring in relation to population density in order to increase the probability of their offspring dispersing during periods of high population density.
- 2) masculinity and sex ratio should be positively correlated under the supposition that if producing males increases a dam's fitness, then an alternate way to increase fitness would be to produce masculine individuals.

THESIS OVERVIEW

This thesis contains a total of four chapters, including this introductory chapter. In Chapter II, "Plasticity of maternal care in a wild rodent is associated with changes in population density", I quantify maternal care in wild woodland deer mice, determine whether wild individuals display the same behaviours as laboratory rodents, and demonstrate that changes in maternal care are associated with changes in population density throughout one breeding season. In Chapter III, "Seasonal adjustment of sex ratio and offspring masculinity by female deer mice is inconsistent with local resource

competition”, I investigate sex ratio and population density fluctuations within a 16-year study on woodland deer mice to determine whether local resource competition occurs in this system. I also investigate whether masculinity is correlated with population density and litter sex ratio in the context of local resource competition. Chapter IV provides general conclusions for Chapters II and III, and suggests future research directions.

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CHAPTER II

PLASTICITY OF MATERNAL CARE IN A WILD RODENT IS ASSOCIATED WITH CHANGES IN POPULATION DENSITY

INTRODUCTION

VARIATION IN MATERNAL CARE

When nurturing offspring a mother may transmit information about the current environment to the subsequent generation (Mousseau and Fox 1998). This form of preparing offspring for potential conditions they might encounter is termed a maternal effect and represents a form of transgenerational phenotypic plasticity (Bernardo 1996; Mousseau and Fox 1998). In mammals, maternal effects are particularly relevant due to their long period of offspring dependence after birth (Reinhold 2002; Maestripieri and Mateo 2009) during which these effects can be transmitted in the form of maternal care (Meaney 2001; Champagne et al. 2003a; 2003b; Macri and Würbel 2006).

Extensive laboratory studies on rodents have identified many important behavioural, physiological, neuronal, and epigenetic consequences of individual differences in maternal care (Francis et al. 1999; Meaney 2001; Champagne et al. 2003a; Champagne 2009). These studies demonstrate that maternal care varies among dams, but is consistent within a dam, and can be termed a maternal style (Fairbanks 1996). Maternal styles are associated with numerous offspring physiological changes such as changes in blood pressure (Myers et al. 1989a), hormone receptors and number of dendrites within the brain (Weaver et al. 2004; Sapolsky 2004; Hasselt et al. 2012; Labonte et al. 2012), stereotypic behaviours (Bechard et al. 2012), and the stress response (Caldji et al. 2000;

Francis et al. 2002). They are also associated with the epigenetic transmission of maternal care between generations (Weaver et al. 2004; Champagne and Curley 2005; Champagne 2011). These maternal effects appear to be closely linked to the frequency of nursing and licking/grooming behaviour received by offspring within the first few days of life (Weaver et al. 2004). These two tactile behaviours have the potential to regulate hormone receptor gene expression through DNA methylation, which impacts the offspring's behaviour through the regulation of hormone receptors within the brain (Weaver et al. 2004; Champagne 2009; Champagne 2011). In stable environments, these associated neuronal changes are stable throughout an individual's life, and are transmissible across generations, from mother to daughter, in the form of nursing and/or licking/grooming behaviours (Francis et al. 1999; Weaver et al. 2004; Champagne 2011).

Although laboratory rodents are consistent in their maternal care across litters, there is still behavioural variation on a finer scale. For example, dams display behavioural plasticity throughout each night and over the first six postnatal days (Priestnall 1972; Meaney 2001; Champagne et al. 2003a; Champagne et al. 2007). Within each night the proportion of time spent nursing and licking/grooming is dependant on each rodent strain, whereas across the first six postnatal days, the proportion of time spent nursing and licking/grooming typically decreases (Champagne et al. 2007; Table 2.1). This behavioural plasticity is consistent within a dam across lactation days, but differs among dams (Champagne et al. 2003a; 2003b; Weaver et al. 2004).

While the neurological and behavioural consequences of differences in maternal care have been well detailed in laboratory individuals, ultimate explanations for the maintenance of variation in maternal behaviour require that the fitness consequences of

differences in maternal behaviour be examined under natural conditions. This is not possible in laboratory studies because of the consistent and manufactured laboratory environment. Instead, studies examining how environmental variations affect maternal behaviour and its fitness consequences are needed to explain the maintenance of this phenotypic variation.

Transgenerational phenotypic plasticity occurs when the maternal environment is ‘translated into phenotypic variation in the offspring’ (Mousseau and Fox 1998), and maternal behaviours have been hypothesized to promote adaptive transgenerational plasticity where the mother influences the offspring’s phenotype to match the environment it will experience (Francis et al. 1999; Kaiser and Sachser 2005). This transgenerational plasticity could ‘allow animals to adapt defensive systems to the unique demands of their fluctuating environment’ (Würbel 2001). It is not yet clear, however, whether this behavioural variation and adaptive plasticity also exists in a wild population (Mateo 2007). Developing a wild rodent model would allow us to test hypotheses about adaptive transgenerational plasticity acting through maternal care in a species that experiences natural environmental fluctuations throughout their lifetime.

WOODLAND DEER MICE

As a first step toward understanding the maintenance of behavioural variation in maternal care I quantified variation in maternal care in a wild rodent, the woodland deer mouse (*Peromyscus maniculatus gracilis*). Many traits of *P. maniculatus* vary depending on geographic location (Millar 1984), but woodland deer mice generally live in mature deciduous forests of North America (Svihla 1932; Hamilton 1941; Wolff and Hurlbutt

1982) and experience wide environmental variation both within and between breeding seasons (Hamilton 1941; Wolf et al. 1985; Ostfeld et al. 1996; Falls et al. 2007).

Detailed quantitative behavioural studies in *Peromyscus* are scarce, however, it is known that mothers commence maternal behaviour directly after birth by first licking their pups (Rheingold 1963). Subspecific variation occurs in nest attendance duration (14 to 20 hrs/day) and frequency (Hill 1972), pup retrieval times (24 to 156 s; King 1963), and the degree to which offspring cling to their mother's nipples (18 to 100 % of littermates; Rheingold 1963). It appears that *P. m. gracilis* has the highest nest attendance of any subspecies (Rheingold 1963), however, the exact maternal behaviours occurring in the nest, how these behaviours change over the lactation period, and the variation in these behaviours within a population are currently unknown.

In this study I quantified variation in maternal care in *Peromyscus maniculatus gracilis*, following well established protocols for measuring maternal care in laboratory rodents (Myers et al. 1989a; Champagne et al. 2003b; Hager and Johnston 2007). I captured pregnant females in their natural environment throughout one breeding season and temporarily housed them in captivity. Their maternal provisioning, maternal responsiveness, and maternal behaviour were quantified within each night and across the first six postnatal days. Quantifying maternal care in wild *P. maniculatus* offers the opportunity to integrate laboratory and field studies by implementing laboratory protocols on wild individuals who might experience similar genetic and epigenetic changes as laboratory individuals (Shorter et al. 2012), but also experience natural environmental variation. Developing a wild rodent model for maternal behaviour also provides the

opportunity to study factors promoting the maintenance of variation in maternal behaviour in the future.

METHODS

STUDY SPECIES AND SITE

Research was conducted in Algonquin Provincial Park, Ontario, Canada (45°37'N, 78°21'W) during August 2010, and May through September 2011. Deer mice were captured using Longworth live traps (13.8 cm x 6.4 cm x 8.4 cm) on both a 16 ha trapping grid and various 100 m transects. Traps were placed at 20 m intervals throughout the grid or at 10 m intervals on transects. Traps were baited with water-soaked sunflower seeds (*Helianthus* spp.) and polyester bedding at dusk, and then checked at dawn. Upon capture weight was recorded using a Pesola spring scale to the nearest 0.5 g, and each individual was marked with two metal ear tags (National Tag and Band Co.). Each individual's age was assessed by dorsal pelage, where juveniles were grey, subadults a mixture of grey and brown, and adults completely brown (Schug et al. 1991). Female reproductive condition was assessed as non-perforate, perforate, or pregnant, and nipples were noted as either non-lactating or lactating.

LABORATORY PROCEDURES

Animal husbandry

Captured females were transported to the animal facility at the Algonquin Wildlife Research Station for maternal observations. Females were housed in a contained and

animal-proof laboratory room with natural diel light hours, temperature, and humidity conditions and kept in individual transparent Plexiglass cages (35 cm x 15 cm x 17 cm) with ad lib access to food (2014 Rodent Chow 14% protein) and water. Corncob (Harlan Corn Cob bedding #7092,), paper (Andersons CN Crink-l'nest™), and cotton bedding (Ancare Nestlets) were provided, as well as a toilet paper roll for environmental enrichment (Würbel et al. 1998). These were replaced once every three days in accordance with SOP-AH3i guidelines for mice, except when pups were present in the cage. After parturition, pups were sexed and weighed to the nearest 0.01 g using an electronic balance, after which mothers and pups were left undisturbed for the remainder of the maternal observation period.

Females who were not gaining weight or visibly pregnant were released after one week in the laboratory. Three females in 2010, and 32 females in 2011 gave birth in the laboratory with one case of infanticide. This resulted in 34 litters for which there were complete maternal observations.

Maternal observations

Our protocol for assessing maternal behaviour followed previous behavioural and physiological protocols for laboratory rats (*Rattus*) and mice (*Mus*) (Priestnal 1972; Myers et al. 1989a, Champagne et al. 2003a; Frances Champagne pers. com.). During the first six postnatal days, dams and pups were video recorded under red light conditions at night, as this is when mice are most active. Cages were not disturbed during these six days. Video recording began at dusk and ran for the duration of the dark phase for each

day. All recordings were made using a SONY HANDYCAM (MODEL #DCR-SR68) video camera.

For each of postnatal days one through six, I collected behavioural data from four pre-designated 1-hour focal periods that were evenly spaced throughout the dark phase of each 24-hour period. The exact focal times changed throughout the sampling period (May through September) due to the change in daylight hours during the summer months. However, two focal periods always began before midnight (designated the “early” focal periods), and two after midnight (designated the “late” focal periods). Each focal period was 60 minutes in duration, and no focal took place within one hour after dusk or one hour before dawn.

Maternal provisioning

On the seventh day postpartum, dams and pups were subjected to a maternal provisioning test (Hager and Johnstone 2007). This test represented a second measure of maternal behaviour. Dams were initially removed from their natal cage at 10:00 hrs and placed into a separate cage with fresh bedding and ad libitum access to food and water. At this point the pups remained in the natal cage, and the mother and pups were separated for four hours. Just prior to the end of this four-hour separation, pups were weighed to the nearest 0.01 g, sexed, given a unique ear notch for that litter, and several measurements were taken; tail length (mm), body length (tail plus body; mm), hind foot length (mm), and ano-genital distance (mm). At this point dams were also weighed.

At 14:00 hrs dams were reunited with their pups in the natal cage for a period of two hours during which time dams had no access to food or water. The next two hours

were video recorded and later analyzed for the amount of time that the dam spent nursing her pups. At the end of two hours the dam and pups were weighed again and the weight change over the two-hour nursing period of dams and each individual pup was noted. Litter weight gain (g) was used as a maternal provisioning value. After weighing, dams were returned to their pups in the natal cage and once again had ad libitum access to food and water.

Retrieval test

At 22:00 hrs on the seventh day postpartum dams and pups were subjected to a pup retrieval test (Champagne et al. 2007). This test was our third measure of maternal behaviour and quantified the responsiveness of the dam to her offspring. The dam was momentarily removed from the natal cage for approximately one minute. Three pups were randomly selected from the litter, removed from the nest, and placed in the opposite corner of the cage from the nest. Any additional pups in the litter were removed from the cage, placed in a separate cage with clean corncob bedding, and placed on the opposite side of the laboratory from the pup retrieval cage (a minimum distance of 4 meters). Dams were then returned to the nest in their home cage, and the latencies (s) for dams to sniff the bedding, retrieve the 1st pup, retrieve the 2nd pup, retrieve the 3rd pup, and start to re-build their nest were noted. If a female had not completed any of these tasks within 15 minutes the test was ended. All offspring were then returned to the nest within the natal cage.

All laboratory procedures were approved under the University of Guelph Animal Care Committee (AUP# 08R063). Maternal observations, maternal provisioning tests, and retrieval tests were conducted within a female's home cage in the laboratory.

DATA EXTRACTION

One primary observer and four secondary observers extracted behavioural variables from video footage of each female recorded between postnatal days one through six. Within each 1-hour focal period maternal behaviour was scored every three minutes (21 observations/focal x 4 focals/night x 6 post natal nights = 504 observations/dam). At each three-minute interval dams were first scored on their location in the cage: 1) 'in nest', where they were in physical contact with nesting material, or 2) 'out of nest', when not in contact with nesting material. Maternal behaviours were not mutually exclusive, but were scored as the following: 1) blanket nursing, 2) arched-back nursing, 3) passive nursing, 4) licking/grooming pups, 5) nest-building, 6) non-nest building, 7) self-grooming, 8) eating, 9) drinking, 10) sniffing, 11) rear/climbing, 12) resting, 13) carrying pup, 14) out of sight (Table 2.2) (Myers et al. 1989a, Champagne et al. 2003a). Nursing postures were often concealed by the bedding material and as a result were difficult to delineate in *Peromyscus*, so the sum of the three observed postures (blanket nursing, arched-back nursing and passive nursing) was described simply as nursing, and was used in our analyses as a single overall measure of maternal behaviour.

STATISTICAL ANALYSIS

In order to determine if there was consistent individual variation in maternal behaviour in *Peromyscus* I quantified each mother's behaviour within each postnatal night, across six postnatal days, and over the breeding season. I used linear mixed effect models to test for significant variation among individuals in 1) their proportion of time spent nursing, 2) in how they changed the amount of time they spent nursing from focals early in the night (before midnight) to later in the night (after midnight), and 3) how they changed their nursing behaviour over the first six post natal nights. Entering 'individual' as a random effect in a mixed-effect model allowed me to quantify the among individual variation in nursing behaviour (repeatability). This linear mixed effects model included proportion of time spent nursing as the response, focal time as a fixed effect to account for differences in nursing behaviour between early and late focal periods, and a random effect for individual intercept. I used the among-female variance and the residual variance from this model to calculate repeatability as the ratio of among individual variation to the total individual variation.

I then investigated how individual females changed their proportion of time spent nursing over one night. To do this, I added a second random effect to the model above that estimated the variance among females in their change in the proportion of time spent nursing from early in the night until later. This method is known as the random regression model (*sensu* Nussey et al. 2007). I assessed the significance of this second random effect by comparing the fit of this model to the fit of the simpler model that included only a single random effect using a likelihood ratio test. This likelihood ratio test determines whether the inclusion of the second random effect for the variance among females in the

slope significantly improved the fit of the model to the observed data. I interpreted significant differences between these two models as evidence of individual differences in how behaviour changed over the course of a night.

I lastly examined the correlation between the individual random effects for intercept and slope. This correlation demonstrates how an individual's average amount of nursing was correlated with its change in nursing behaviour over the course of the night. A negative correlation would demonstrate that females that on average nursed more also decreased their nursing over the course of the night, whereas a positive correlation would demonstrate that females that nursed less than average nursing dams increased their nursing over the course of the night. I then performed the exact same three step analyses outlined above for nursing behaviour across the first 6 postnatal days by replacing the random effect for individual slope within each night with the random effect for individual slope across post natal days.

I tested for the presence of a maternal style in this population of wild *Peromyscus* by examining correlations between maternal behaviour, maternal provisioning, and maternal retrieval. Because of seasonal changes in maternal behaviour (see results) I determined the covariation between maternal variables by correlating the residuals of the relationship between the proportion of time spent nursing and Julian parturition date with either maternal provisioning (g) or maternal responsiveness (latency to nest build; s).

To determine whether maternal care was repeatable across litters I correlated the proportion of time spent nursing in the first observed litter against the proportion of time spent nursing in the second observed litter for the four dams who delivered two litters in the lab.

In wild populations, changes in the mean value of a trait through time can be explained by either a change in the composition of the population, or by individual phenotypic plasticity (Przybylo et al. 2000). Laboratory studies have demonstrated that maternal behaviour is lab rodents repeatable (Francis et al. 1999; Champagne et al. 2003a; 2007; Weaver et al 2004), but they typically do not directly investigate the plasticity of maternal behaviour. I investigated seasonal plasticity of maternal behaviour by regressing the proportion of time spent nursing against the Julian date on which the litter was born. I further tested whether seasonal changes in maternal behaviour could be explained by individual plasticity by comparing the average magnitude of individual plasticity to the magnitude of seasonal trait change observed in the population (sensu Przybylo et al. 2000). I measured the average magnitude of individual plasticity by calculating the change in the proportion of time spent nursing between successive litters, divided by the number of days between the production of these litters for each repeat female. These individual values were averaged across the four females for which I quantified maternal behaviour in two litters within 2011 and I calculated 95% CIs around this mean individual plasticity. I compared the slope and 95% CIs from the population change model to the average individual plasticity to determine whether the seasonal change in maternal behaviour could be explained by individual plasticity in maternal behaviour (sensu Przybylo et al. 2000).

Individuals in a wild population are subjected to changes in environmental variables throughout a breeding season, and most wild populations of small mammals exhibit seasonal population growth. Densities are lowest over winter, increase in spring, and peak during the fall (Fretwell 1972; Boyce 1979; Eccard and Rödel 2011). I

determined whether there was any association between maternal behaviour and population density in *Peromyscus* using population density data from an ongoing long-term small mammal survey that was conducted nearby. Density was estimated for each of eight two-week intervals throughout the summer as the number of captures per 100 trap nights from seven transects situated in maple hardwood and cut-over mixed wood stands, which represented similar habitats to the trapping locations of animals used in this study. Each density transect contained 10 pairs of traps spaced at 10 m intervals (Falls et al. 2007). Density transects were located within 25 km (range: 0.5 to 25 km) of the capture locations of the females used in this study and mouse population dynamics in the area have been shown to respond to wide regional variation in food availability (Falls et al. 2007). I also investigated the correlation between population density in the wild and the number of mice that were housed in the laboratory during maternal behaviour tests during the same two-week periods. To determine whether maternal behaviour was associated with either population density or housing density, generalized linear models were used where the average proportion of time spent nursing was modeled with either the average density or number of mice present in the laboratory in each two-week period.

All statistical analyses were performed in R version 2.13.2 (R Development Core Team 2011) using the lme4 package (Bates et al. 2011) with either a binomial link (for binomial data) or a Poisson link (for count data) where necessary, or the nlme package (Pinheiro et al. 2011). Statistical significance of models did not change when analyzed in either package. Inter-rater reliability was calculated as a correlation of behaviour scores

by a primary observer against the mean value from secondary observers. All values are presented as means \pm s.e.m. unless otherwise stated.

RESULTS

VARIATION IN MATERNAL BEHAVIOUR

Maternal behaviours in *P. maniculatus* were similar to behaviours observed in laboratory rodents and varied over each focal night, over the lactation period, and between individuals. Thirty-four dams spent on average $39 \pm 24\%$ of their time in the nest and $61 \pm 25\%$ of their time out of the nest. The majority of their time was spent either nursing ($41 \pm 25\%$), rearing/climbing ($29 \pm 20\%$), or eating ($12 \pm 8\%$), but other behaviours that occurred were sniffing air ($4 \pm 5\%$), resting ($4 \pm 5\%$), licking/grooming ($4 \pm 5\%$), self-grooming ($3 \pm 4\%$), nest building ($2 \pm 4\%$), drinking ($0.8 \pm 1\%$), non-nest building ($0.05 \pm 2\%$), carrying pup ($0.01 \pm 5\%$), or out of sight ($11 \pm 19\%$; Figure 2.1). Note that percentages do not sum to 100 % because some behaviours were not mutually exclusive (e.g. females could be eating while nursing). The proportion of time spent nursing over the entire sampled population was normally distributed and had an inter-rater reliability of 86%.

Individual repeatability for nursing behaviour within a night was low ($R = 0.20$; Figure 2.2). The likelihood ratio test between two models without fixed effects, one with a random effect for individual intercept and the other with a random effect for both individual intercept and slope, demonstrated that the random effect for individual slope was significant ($X^2_1 = 7.11$, $p = 0.03$), which indicates that there were significant

differences among individual females in how their behaviour changed between early in the night to later in the night. Finally, the correlation between both random effects (individual intercept and slope) was -0.82, which demonstrates that higher than average nursing dams decreased their nursing within each night. This led to a decrease in individual variation in nursing values between early and late focal periods (Figure 2.2).

Individual repeatability for nursing behaviour across postnatal days was low ($R = 0.18$; Figure 2.3). The likelihood ratio test between two models without fixed effects, one with a random effect for individual intercept and the other with a random effect for both individual intercept and slope, demonstrated that the random effect for individual slope was significant ($X^2_1 = 21.29$, $p < 0.001$), which demonstrates that there were significant differences in how individuals changed their behaviour over the first six postnatal days. Finally, the correlation between both random effects (individual and post natal day) was -0.28, which demonstrated that higher than average nursing dams slightly declined their nursing over post natal days. This did not result in an obvious decrease in individual variation in nursing values across postnatal days (Figure 2.3).

Maternal provisioning (change in total litter weight) ranged between -0.12 g to 1.18 g and averaged 0.63 g, whereas maternal retrieval (latency to nest build) averaged 388 ± 58 s in duration. The proportion of time spent nursing was not significantly correlated to maternal provisioning ($r = -0.17$, $p = 0.35$) or maternal responsiveness ($r = 0.19$, $p = 0.31$), and maternal provisioning and maternal responsiveness were not correlated with the Julian date on which the litter was born (provisioning: $r = 0.01$, $p = 0.94$; responsiveness: $r = 0.02$, $p = 0.90$). The proportion of time spent nursing decreased over the breeding season (slope = -0.002, $n = 30$, $R^2 = 0.25$, $p = 0.003$) and the residuals of this

relationship were also not correlated with either maternal provisioning ($r = -0.20$, $p = 0.27$) or maternal responsiveness ($r = 0.25$, $p = 0.18$).

CONSISTENCY OF MATERNAL BEHAVIOUR

Repeatability of maternal behaviour

The proportion of time spent nursing was highly repeatable when observations from the first litter were correlated against observations from the second litter ($r = 0.97$; $p = 0.02$; Figure 2.4).

Plasticity of maternal behaviour

Among all litters, the proportion of time spent nursing significantly decreased over the course of the breeding season (slope = $-0.002 \pm 95\% \text{ CL} = 0.0022 \pm 0.0013$, $n = 30$, $R^2 = 0.25$, $p = 0.003$; Figure 2.5a). This was extremely similar to the average magnitude of individual plasticity for the four individuals that bred twice (slope = $-0.002 \pm 95\% \text{ CL} = 0.0027 \pm 0.0014$, $n = 4$; Figure 2.5b). The similarity in slope and extensive overlap in 95% CIs for these two models suggests that the observed seasonal change in maternal behaviour can be explained by individual plasticity in maternal behaviour.

Maternal behaviour and density

Population density in the area increased from 5 captures/100 trap nights in May to 44 captures/100 trap nights in August. During this time the average proportion of time spent nursing declined and there was a significant negative effect of population density on the

proportion of time spent nursing over the eight two-week periods of the summer ($n = 8$, $R^2 = 0.56$, slope = -0.006 ± 0.002 , $p = 0.02$) (Figure 2.6). Estimated population density and the number of dams in the laboratory were also correlated ($r = 0.92$, $df = 7$, $p < 0.001$), but the effect of the number of dams in the laboratory on the proportion of time spent nursing was only marginally significant ($n = 8$, slope = -0.014 , $R^2 = 0.34$, $p = 0.07$).

DISCUSSION

Here, we quantified variation in maternal care in a wild rodent and demonstrated that laboratory and wild individuals display similar behaviours but differ in their behavioural plasticity. Wild and laboratory mice both provide tactile stimuli to their offspring through nursing, however, they differ in their frequency of this behaviour. *Peromyscus* individual variation in nursing spanned the range in variation seen across strains of *Mus*, where *Peromyscus* spent between 15 to 64% of their time nursing and *Mus* strains spent between 49 to 67% of their time in the arched-back nursing position alone (Champagne et al. 2007). Within this study, wild dams spent the majority of their time either nursing, rearing/climbing, or eating, and spent less than 5% of their time in any of the other behaviours. Individual variation in nursing declined between early and late focal periods but was maintained across postnatal days. This wild population of *P. maniculatus* demonstrated a much larger magnitude of individual behavioural variation than laboratory studies on maternal care (Würbel 2001; Weaver et al. 2004; Champagne et al. 2007).

Laboratory mice (Champagne et al. 2003a; 2007) and wild mice are similar in that they both display repeatable maternal behaviours, which comprise an offspring's early maternal environment. The concept of an early maternal environment can be applied across mammalian species, and although the exact behaviours differ between species, the early maternal environment in general has been cited as an important aspect regulating the frequency of anxiety and depression (Pruessner et al. 2004; Sroufe 2005; Champagne 2010), diabetes and cardiovascular disease (Baten et al. 2004; Goodwin and Stein 2004), susceptibility to drug abuse (Dube et al. 2003; Dube et al. 2006), and domestic violence (summarized in Champagne and Curley 2010) in humans. In wild *Peromyscus*, individuals demonstrated a high level of repeatability between litters ($r = 0.97$), but the repeatability of individuals was low when looking at behavioural variation within a night ($R = 0.20$) and across postnatal days ($R = 0.18$). Nursing behaviour parallels some laboratory strains by declining within a night (Champagne et al. 2007; Figure 2.2) but contrasts with laboratory strains by remaining relatively constant over postnatal days (Champagne et al. 2007; Figure 2.3). Over the breeding season nursing behaviour in *Peromyscus* was demonstrated to be plastic but also repeatable, where the rank order of individual females remained the same regardless of Julian date (Figure 2.5b). Although an individual's behaviour may decline over the breeding season, it remains in its relative rank in comparison to the rest of the population at any particular Julian date. This consistency in individual maternal behaviour parallels laboratory results of maternal care on mice (*Mus*; Champagne et al. 2007), rats (*Rattus*; Weaver et al. 2001), guinea pigs (*Cavia aperea f. porcellus*; Albers et al. 1999), and striped mice (*Rhabdomys pumilio*; Rymer and Pillay 2012). However, these results in *Peromyscus* suggest that early

maternal environments may also be naturally variable in other wild species, raising the question of whether adverse early environments absolutely result in the negative consequences described in the above human studies.

We observed a large decrease in the proportion of time spent nursing across the breeding season (Figure 2.5a,b). Population level changes in phenotype could be due to either individual plasticity or a change in the composition of the population (Przybylo et al. 2000). That we saw the same level of decrease in nursing across the breeding season by individual repeat-breeders as in the population as a whole indicates that phenotypic plasticity is a sufficient explanation of our observed seasonal change in maternal behaviour in this wild rodent. However, we know little of whether such plastic responses are adaptive.

Behavioural plasticity that co-varies with environmental variation implies that the environment might have a profound effect on behavioural phenotype. Wild individuals experience a variety of environmental conditions during their lifetime, however, population density often fluctuates both within and between years and the behaviour of individuals is often cited as a key component of density-dependent population regulation (Chitty 1967; Krebs 1978, 1996; Sinclair 1989; Mougeot et al. 2003). Few studies directly link density-dependent behaviour to population density, but some have demonstrated an effect of individual behaviour on population level processes (Kokko and López-Supulcre 2007). Experimental manipulation of population density has demonstrated that density influences feeding and foraging rates (Dobbs et al. 2007; Rutten et al. 2010) as well as offspring provisioning rates (Sillett et al. 2004; Bretagnolle et al. 2008) in wild animals. Dantzer et al. (2012) demonstrated that North American red

squirrels (*Tamiasciurus hudsonicus*) have lower nest attendance when perceived population density was increased, but it is not clear how female red squirrels are behaving while in the nest with their pups. The cue that allows individual deer mice to assess changes in population density is elusive, however, similar to female red squirrels, behavioural adjustments may occur in *Peromyscus* if individuals are able to sense a change in population density.

The reasons for the observed change in maternal behaviour with population density are currently unknown. In laboratory rodents, manipulation of maternal behaviour has been shown to influence the behavioural phenotype of offspring through physiological mechanisms, specifically the offspring stress response (Caldji et al. 2000; Francis et al. 2002), alterations in blood pressure (Myers et al. 1989a), and alternations to the density of hormone receptors in targeted tissues as well as the number of dendrites within the brain (Weaver et al. 2004; Sapolsky 2004; Hasselt et al. 2012; Labonte et al. 2012). For example, Myers et al. (1989a; 1989b) demonstrated that maternal care influences the development of the cardiovascular system, where a decrease in maternal care is reflected in a decreased blood pressure of adult offspring. The adaptive benefit of adjusting offspring phenotype and physiology through maternal care is speculative, but physiological adjustment such as a decrease in blood pressure, may be adaptive for late-season environmental conditions. Additionally, Eccard and Rödel (2011) determined that aggressiveness changed with seasonality in European Rabbits (*Oryctolagus cuniculus*), where they reported that offspring behavioural plasticity is dependent on litter size and parturition date. In wild *Peromyscus*, maternal behaviour may decrease over the breeding

season as an adaptive mechanism whereby mothers adjust offspring phenotype to aid offspring survival.

Maternal behaviour, and maternal styles in general (Fairbanks 1996; Sapolsky 2004) can affect offspring phenotype in other systems (Smotherman and Bell 1980; Francis et al. 1999; Weaver et al. 2001; Champagne et al. 2003a; Dube et al. 2006; Danchin et al. 2011). There was no apparent maternal style in this study, and the direct association between maternal behaviour and offspring phenotype has not been determined in *Peromyscus*. However, one hypothesis of the seasonal change in maternal behaviour is that maternal care late in the season might better prepare offspring for over-winter survival. *Peromyscus* live in seasonal environments and are active throughout the winter (Svilha 1932; Hill 1972); however, they may experience extended periods of extreme cold or food shortages (Wolff and Hurlbutt 1982; Wolff et al. 1985). Many physiological examples exist of individuals changing their phenotype to prepare for periods of hibernation, torpor, or food shortages, including increasing insulation through pelage or fat deposits, vasoconstriction, decreased respiration and metabolic rates, and changes to endocrinology (Moyes and Schulte 2006). There is current interest in the field of epigenetics regarding seasonal adaptations, where it has been proposed that there may be individual-based epigenetic changes in specified organs between summer and winter months (Fujii et al. 2006; Storey et al. 2010). Maternal care can induce epigenetic changes in laboratory rodents (Weaver et al. 2004), and epigenetic changes associated with agouti coat expression, and potentially stereotypies, have been reported in *Peromyscus* (Shorter et al. 2012). However, it has yet to be seen if these mechanisms apply to *Peromyscus* regarding maternal care. Whether through endocrine function or

another mechanism, the observed change in maternal behaviour in this wild study may represent one way by which dams are programming their offspring's phenotype for a seasonal environmental change.

The observed plasticity in maternal behaviour in wild *Peromyscus* over the breeding season highlights that maternal behaviour might change in response to changes within the environment. Multiple years of data are required to determine whether the observed changes in maternal care are consistent across years and also to evaluate if maternal effects confer an adaptive advantage. This viewpoint does not dichotomize maternal care as either positive or negative. Rather it implies that maternal care is one example where mothers alter their behaviour to changes in environmental conditions. Placing the observed phenotypic plasticity of maternal care into an evolutionary perspective builds on the important contributions of laboratory studies by investigating the behavioural variation in maternal care in an appropriate environmental context , and emphasizes that maternal care in wild individuals is both repeatable and highly dependent on the environment.

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TABLES

Table 2.1 – Summary table comparing attributes of strains of laboratory mice (*Mus*) to individual wild woodland deer mice (*Peromyscus maniculatus*). *Peromyscus* demonstrate more variable nursing behaviour in all attributes measured when compared to *Mus*.

Attribute	Laboratory <i>Mus</i> Strains	Citation	Wild <i>Peromyscus</i> individuals
Nursing behaviour	Frequency of nursing between 49 to 67% depends on strain	Champagne et al. 2007; Curley et al. 2009; Hager and Johnstone 2006	Frequency of nursing between 2 to 64% for individuals
Nursing within one night	Changes in mean frequency of nursing is strain dependant; some strains increase nursing within one night while others decrease nursing	Champagne et al. 2007	Variation in nursing between individuals decreases within a night
Nursing across nights	All strains decrease nursing across postnatal nights	Champagne et al. 2007	Individuals differ in how their nursing changes across postnatal nights
Nursing across season	N/A	N/A	Seasonal decrease in nursing is explained by phenotypic plasticity

Table 2.2 - Maternal behaviours of dams during behavioural trials. Location and behaviour was categorized once every three minutes during a 1-hour focal session. Not all behaviours were mutually exclusive.

Location	Description
In nest	Dam is in physical contact with nesting material
Out of nest	Dam is not in physical contact with nesting material

Behavior	Description
Nursing	
1. blanket nursing	Dam is positioned over the pups to permit sucking or thermoregulation with a low to moderate arch in her back
2. arched nursing	Dam is positioned over the pups with a high arch in her back to permit sucking and pup movement
3. passive nursing	Dam is lying on her side with her ventrum exposed to the sucking pups
Licking/grooming	Dam is licking pups (ano-genital or body region)
Nest shavings	Dam is picking up pieces of bedding and retrieving these to the nest or moving bedding in the nest with her snout
Non-nest shavings	Dam is picking up pieces of bedding but NOT retrieving these to the nest
Self-grooming	Dam is licking herself (often occurred during bouts of pup licking)
Eating	Dam is eating
Drinking	Dam is drinking
Sniffing air	Dam is either in or out of the nest sniffing the air or corn bedding.
Rearing/climbing	Dam is out of the nest and is either rearing on her hind legs or climbing and biting on the bars of her cage
Resting	Dam is either in or out of the nest and does not appear to be doing anything (occurs frequently while out of the nest and in one corner of the cage).
Carrying pup	Dam is carrying a pup
Out of Sight	Dam is hidden by bedding or is not visible on video footage

FIGURES

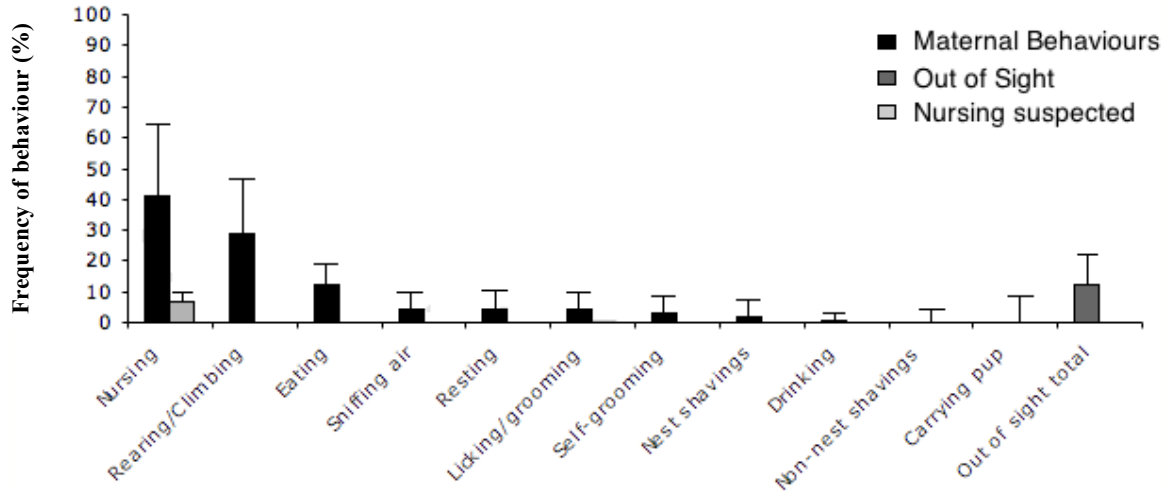


Figure 2.1 - Frequency of behaviours from maternal observations (black). Dams were scored as one of 12 behavioural categories during each focal session. Out of sight behaviours (grey) were included as a non-mutually exclusive behaviour for instances when the behaviour of dams was not visible. Instances where nursing behaviours were suspected but the individual was out of sight (in the case where dams were in the nest but not visible due to nesting materials) occurred in less than 6% of all observations.

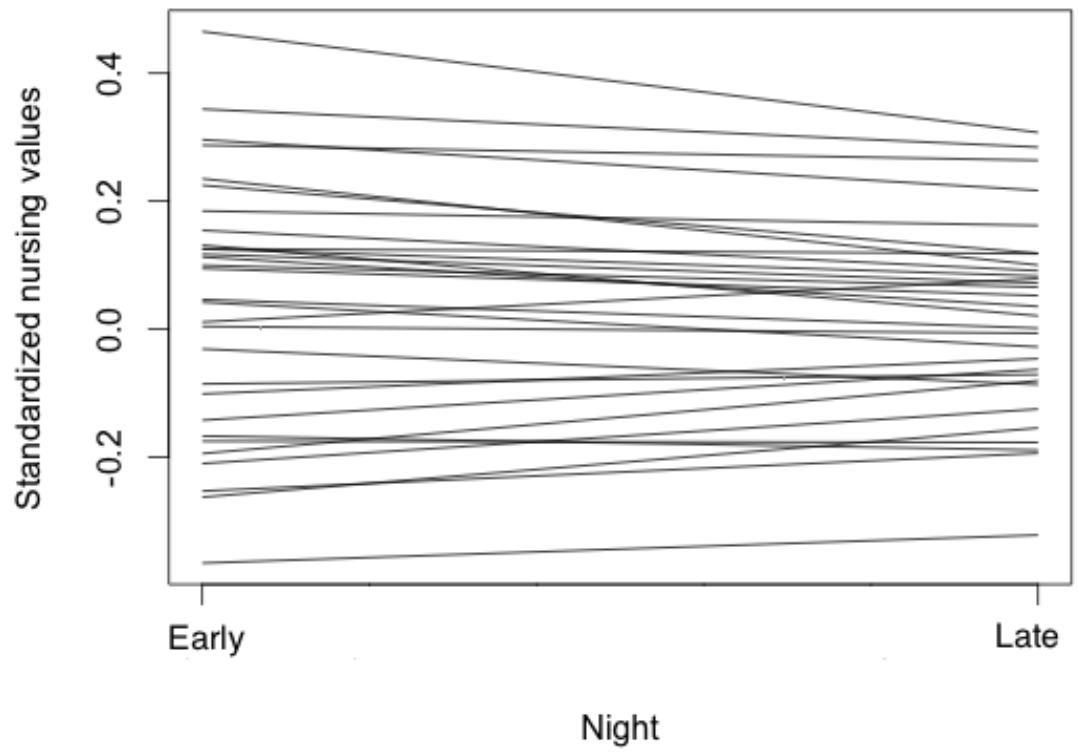


Figure 2.2 - Predicted values for the proportion of time spent nursing throughout a night for individual *Peromyscus* dams. Each line represents one dam (n = 30) and her change in nursing is shown as the slope between the early and late part of the night. Individuals differed in their change in proportion of time spent nursing throughout one night.

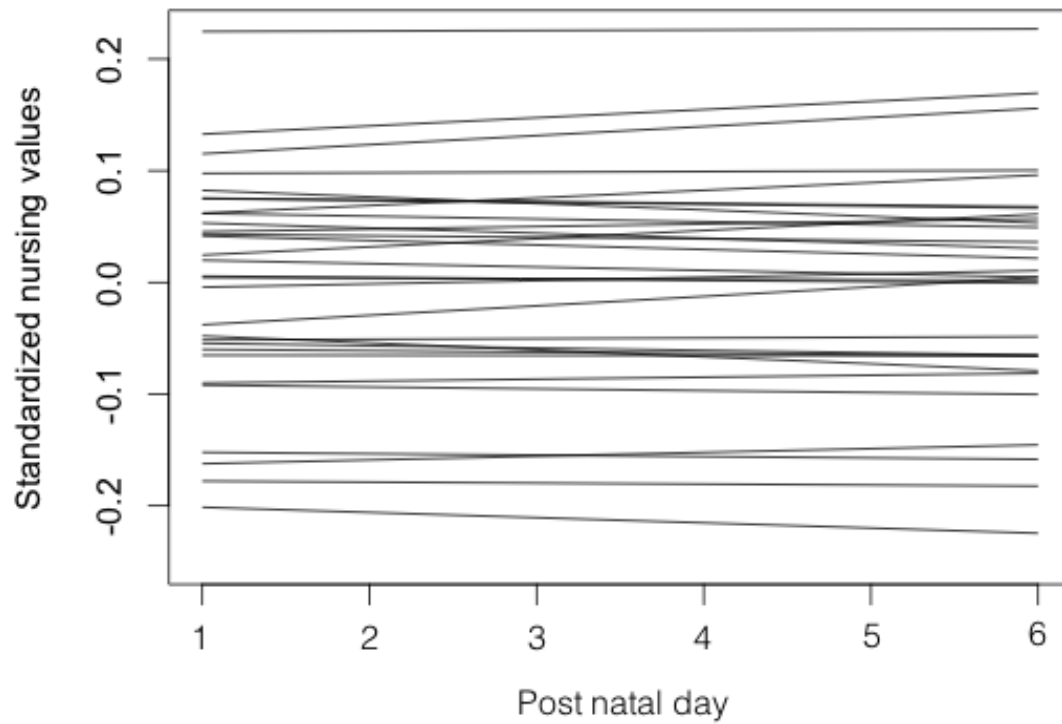


Figure 2.3 - Predicted values for the proportion of time spent nursing throughout post-natal days of individual *Peromyscus* dams. Each line represents one dam ($n = 30$) and her change in nursing is shown as the slope between post-natal day 1 through 6. Individuals differed from one another in their change in proportion of time spent nursing throughout post-natal days.

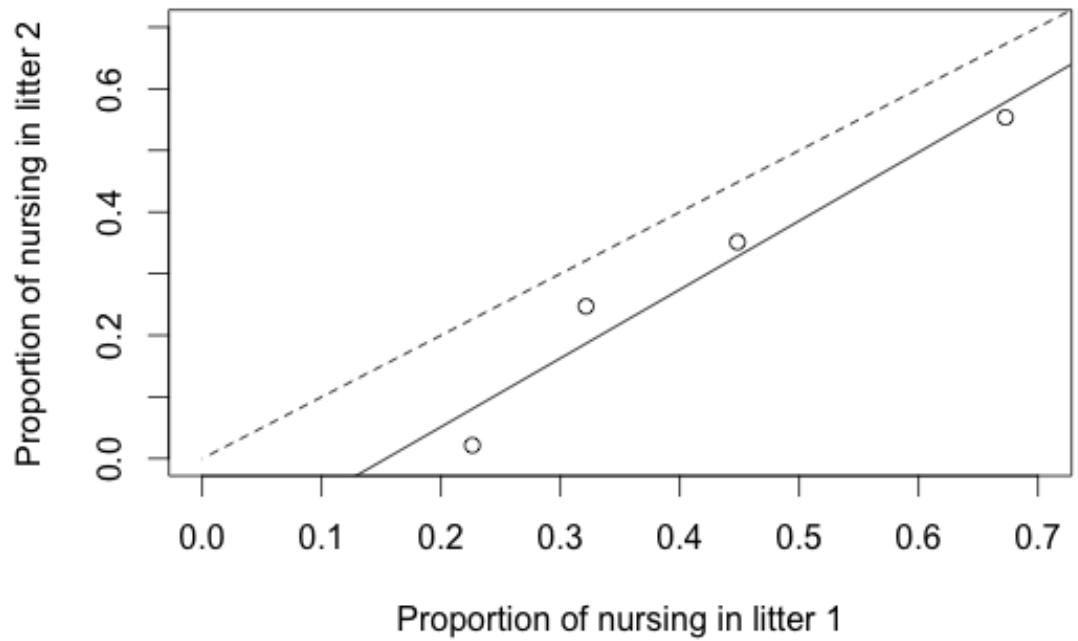


Figure 2.4 - The proportion of time spent nursing in the second observed litter correlated with the proportion of time spent nursing in the first observed litter for the four females that produced two litters (solid line). The 1:1 line is represented by the dashed line. The fact that values are consistently below this line indicates that nursing in litter 2 is less than nursing in litter 1. However, the strong correlation between observations (solid line) for successive litters indicates that the proportion of time spent nursing is repeatable between litters.

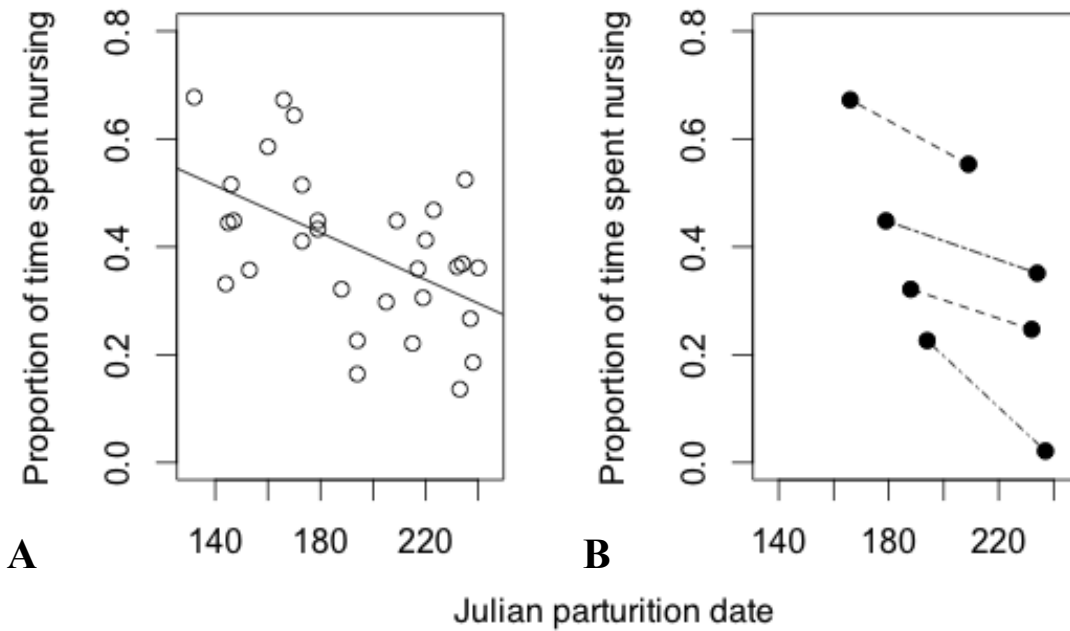


Figure 2.5 - The proportion of time spent nursing by *P. maniculatus* significantly decreased over the course of the season (Julian parturition date). This was true for both the population as a whole (A), as well as for individual females who raised more than one litter during 2011 (B). The average of the individual female slopes in panel B is not significantly different from the population plasticity slope in panel A, indicating that the change in nursing behaviour observed in the population (A) can be explained by individual plasticity in maternal behaviour (B).

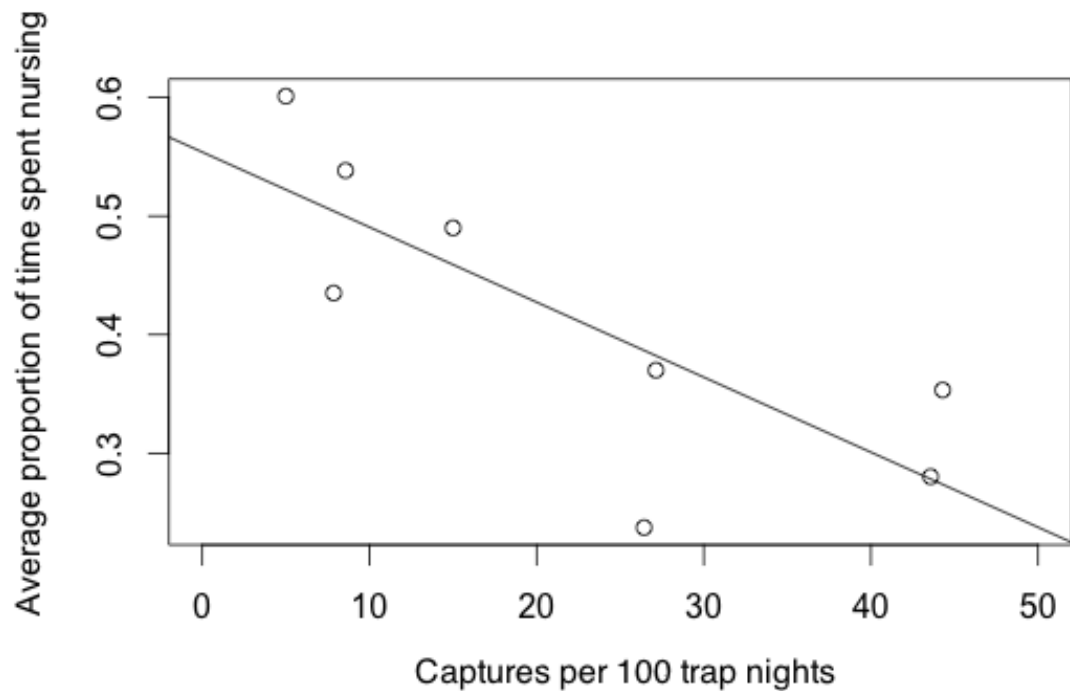


Figure 2.6 - The proportion of time spent nursing by female *Peromyscus* was negatively correlated with the estimated population density at the time they gave birth. Mouse population density was measured for eight two-week periods as the cumulative captures of *Peromyscus* per 100 trap-nights (equivalent to a two week period) in a nearby long-term small mammal study. The proportion of time spent nursing by all dams in this study was also averaged within each two-week period.

CHAPTER III

SEASONAL ADJUSTMENT OF SEX RATIO AND OFFSPRING MASCULINITY BY FEMALE DEER MICE IS INCONSISTENT WITH LOCAL RESOURCE COMPETITION

INTRODUCTION

Theory suggests that parents should generally allocate resources equally towards sons and daughters, leading to an equal sex ratio (Fisher 1930; Clutton-Brock et al. 1982). However, in wild populations, mothers often give birth to biased sex ratios (Sikes 2007). This contradiction has been investigated in detail in several study systems and a variety of current sex ratio theories have made different predictions, including that sex ratio should vary with maternal body condition (Trivers and Willard 1973), philopatry and mating system (Local Resource Competition; Clark 1978; Silk 1983; Sikes 2007), offspring body condition and sexual maturation rate (First Cohort Advantage; Wright et al. 1995; Sikes 2007), or environmentally induced changes to maternal condition (Extrinsic Modification; Post et al. 1999; Forchhammer 2002; Sikes 2007). Sex ratios may vary with the costs of producing high quality male versus female offspring (Clutton-Brock and Iason 1986), but the ultimate goal is to produce as many grand-offspring as possible (Trivers & Willard 1973; Armitage 1987; Silk & Brown 2008; Monclús and Blumstein 2012).

One of the most commonly tested hypotheses about why mothers might adjust offspring sex ratio away from 50:50 is local resource competition (LRC). This hypothesis applies to species that experience variation in population density and exhibit sex-specific dispersal (Clark 1978; Silk 1983; Sikes 2007). In periods of high population density

selection should favour the production of the dispersing sex in order to decrease competition for local resources within the natal territory (Clark 1978). For example, in Belding's ground squirrels (*Spermophilus beldingi*), which exhibit male-biased dispersal (Holekamp et al. 1984; Nunes et al. 1999), local resource competition would predict that more males ought to be produced during periods of high population density. This concept has been both supported and rejected in a variety of taxa, such as small mammals (Goundie and Vessey 1986; Goundie 1986; Armitage 1987; Caley et al. 1988; Lambin 1994a,b,c; Moses et al. 1995; Sikes 1995; Havelka and Millar 1997; Moses et al. 1998; Shibata and Kawamitchi 2009), large mammals (Skogland et al. 1986; Caley and Nudds 1987; Kojola 1998; Fernandez-Llaro et al. 1999; Blanchard et al. 2004; Martin and Festa-Bianchet 2011), primates (Clark 1978; summarized in Silk and Brown 2008), birds (summarized in Gowaty 1993; summarized in Alonso-Alvarez 2003; Rubenstein 2007), insects (summarized in Frank 1985), and plants (Burd and Allan 1988; summarized in Heilbuth et al. 2001).

A generally unrelated body of literature suggests that individual masculinity is also an important component of an individual's phenotype (Moore and Power 1992; Lindstrom 1999; Monclús and Blumstein 2012). The presence of siblings, and sex of those siblings, along with maternal androgens and maternal care have the potential to change the masculinity of an individual (Clemens 1978; Moore 1992; Moore and Power 1992; summarized in Ryan and Vandenbergh 2002; Rosenfeld and Roberts 2004). Anogenital distance (AGD), measured as the distance from an individual's genital papilla to their anus (Ryan and Vandenbergh 2002), is a commonly used measure of masculinity because it is correlated to testosterone levels (Clemens 1974; Ryan and Vandenbergh

2002), is repeatable (Gandelman et al. 1977; Ryan and Vandenberg 2002; Dusek and Bartos 2012), and on average males have a larger AGD than females (Clark and Galef 1990; Ryan and Vandenberg 2002). AGD reflects individual hormonal variation that begins *in utero*, and has consequences on adult behavioural phenotype such as the frequency of aggressive encounters (Monclús et al. 2012), life-history traits such as dispersal distance and age of reproductive maturity, and fitness (Monclús and Blumstein 2012).

AGD measurements are rare in wild animals, so we use examples from several different systems. Cantoni et al. (1999) demonstrated in the California mouse (*Peromyscus californicus*) that AGD of females from male-biased litters was larger than from females of female-biased litters. Drickamer (1996) demonstrated in wild house mice (*Mus domesticus*) that increases in AGD increase the likelihood of dispersal in males, and that there is variation in the probability of dispersal for both sexes, where on average 19% of females disperse and 16% of males disperse (Drickamer 1996). Similar trends in regards to testosterone have been noted in Belding's ground squirrels (Nunes et al. 1999), and yellow-bellied Marmots (Monclús and Blumstein 2012), where experimental manipulation of perinatal testosterone exposure (Holekamp et al. 1984) increased the probability of dispersal among both male and female juveniles (Nunes et al. 1999). Belding's ground squirrels represent a system that experiences drastic variation in population density, sex ratio, probability of dispersal, and masculinity, however, other systems of small mammals may also experience variation in these parameters as well.

Integrating local resource competition and individual masculinity

In general, dams should differentially invest in the sex ratio of their progeny because each sex may differ in their contribution to inclusive parental fitness. The local resource competition hypothesis predicts that dams should vary their production of sons and daughters in relation to population density. The benefit of producing sons when population density is high is because males are more likely to disperse than females. By extension, if more masculine offspring (sons and daughters) are also more likely to disperse, then dams should also vary the masculinity of their offspring in relation to population density. Masculinity might, therefore, provide a fine-scale adjustment to a fitness-related trait (e.g. dispersal probability) that differs between sons and daughters. In this case we would expect a positive correlation between masculinity and the proportion of sons in a litter. This integration of local resource competition and masculinity allows us to move away from predictions that involve discrete variables (ie. male vs. females) and onto a continuum where individuals can be placed anywhere between two opposite extremes (masculine vs. feminine; Figure 3.1).

Woodland deer mice

Woodland deer mice (*Peromyscus maniculatus*) exhibit male-biased dispersal (Fairbairn 1976) and extreme fluctuations in population density (Falls et al. 2007). There is little information as to primary (at conception) sex ratios or individual masculinity in the woodland deer mouse, however, observations from previous studies suggest that secondary (at birth) and tertiary (at first capture) sex ratios may vary with litter size, a mother's physiological condition or Julian date, (Coulson and Hickling 1961; Goundie

and Vessey 1986; Kent 1992; Zuleta and Bilenca 1992), precipitation or temperature (Havelka and Millar 1997), population density, (Canham 1970; Stirling 1971; Kaufman and Kaufman 1982; Myers et al. 1985; Kaufman and Kaufman 1987), or environmental stability (Kempe et al. 2006; Shibata and Kawamitchi 2009). These observations question the assumption that selection should favour a return to equal sex ratios, although the adaptive reasons for these observed variations are still unknown.

Local resource competition may apply to *Peromyscus* where an increase in male-biased litters should be produced during periods of high population density, however, the results of studies investigating sex ratio in *Peromyscus* are varied. Havelka and Millar (1992) found that the shift in tertiary sex ratio differs in direction between seasons in *P. maniculatus* and was correlated to precipitation, but they concluded that this shift was due to differential offspring mortality rather than skewed maternal investment. In the same population of *P. maniculatus*, Millar and Teferi (1992) concluded that resource competition does not occur in this species because there was no reduced survival among large family groups, which would be expected if any form of resource competition were occurring. A shift from male- to female-biased sex ratios has been observed in *P. leucopus* but this shift was correlated to population density, which goes against LRC (Goundie and Vessey 1986). They conclude that the observed shift in sex ratio varies with maternal condition rather than local resource competition.

To date there is limited information on masculinity in *Peromyscus* and the association between AGD, masculinity, and dispersal distance has yet to be investigated. I assume that testosterone is associated to masculinity and dispersal distance in the woodland deer mouse (*Peromyscus maniculatus gracilis*), and use this species as an

example to investigate local resource competition in regards to individual masculinity. I expect that dams might be able to influence the probability of their offspring dispersing by altering individual masculinity rather than offspring sex *per se*.

The goals of this study were twofold. Firstly, I tested the LRC prediction that males should be produced during periods of high population density in a multiparous wild rodent, *Peromyscus maniculatus*. I collected data on *P. maniculatus* from 1996 through to 2011 to test the association between sex ratio and population density. Secondly, I measured AGD during a one-year study on *Peromyscus* (Chapter II) to test my prediction that mothers would produce masculine offspring when population density is high.

METHODS

DATA COLLECTION

Sex ratio

I investigated sex ratio and population density fluctuations of *Peromyscus maniculatus* across 16 years to determine whether females adjusted offspring sex ratio in a manner consistent with the LRC hypothesis. These data were collected from 1996 to 2011 from Algonquin Provincial Park (see Falls et al. 2007), across seventeen 100 m transects placed in eight different forest habitats including coniferous, mixed forest, and pure hardwood stands. Transects were trapped bi-monthly each year between May and August for three consecutive nights (as in Fryxell et al. 1998; Falls et al. 2007).

Population density was calculated as the number of captures (adult, subadult, and juvenile combined) per one hundred trap-nights. Population density was calculated for each year, each spring (May), and each fall (August). Offspring (tertiary) sex ratio was measured as the proportion of young-of-the-year (YY) individuals at first capture that were male and was calculated for each year, each spring, and each fall. YY were considered to be individuals who weighed less than 16 g, where females had a closed vagina and males were non-scrotal, with either grey or mottled grey and brown pelage (Schug et al. 1991).

Masculinity

I collected anogenital distance measurements from offspring of 31 litters that were born in captivity between May and August 2011 at the Algonquin Wildlife Research Station in Algonquin Provincial Park, Ontario, Canada (45°37'N, 78°21'W). Parturition dates of each litter were known from observing dams on a daily basis. After birth offspring were sexed, weighed to the nearest 0.01g, and both body length (cm) and ano-genital distance (cm) were taken at post natal day six using digital calipers (accuracy 1 mm).

Both weight (*Mus* and *Rattus*; Graham and Gandelman 1986; Ryan and Vandenberg 2002) and body length (*Mus* and *Peromyscus*; Drickamer 1996; Hotchkiss and Vandenberg 2005) isometrically covary with AGD in previous studies, and when AGD is divided by one of these parameters, they are termed the ano-genital distance index (AGDI). However, in this study, AGDI (AGD divided by body length) was not a good predictor due to the hypoallometric relationship between $\log(\text{body size})$ and $\log(\text{AGD})$ ($n = 31$, slope = 3.26 ± 0.25 , intercept = -11.96 ± 0.93). I therefore opted

against using AGDI as a measure of masculinity. Instead I simply used AGD and correct for body size by including it as a covariate in my models.

Julian parturition date and proportion of time spent nursing were determined from laboratory observations of dams (see Chapter II). The population density was estimated from 2011 data on the long-term small mammal survey within Algonquin Park and is estimated as captures/100 hundred trap nights, which is equivalent to two-week periods. The time period within my statistical models refers to these two-week periods.

All laboratory procedures were approved by the University of Guelph Animal Care Committee (AUP# 08R063).

STATISTICAL ANALYSIS

Sex ratio

Two methods were used to evaluate the correlation between YY sex ratio and population density; 1) a Pearson's correlation involving data from all 16 years, and 2) a de-trending analysis to account for an effect of year. This de-trending analysis involved the correlation between the residuals of two models; the first model with YY sex ratio as the response with year and year² as fixed effects, and the second model with population density as the response with year and year² as fixed effects. Spring and fall data were compared separately to determine whether sex ratio changed over each year, and how variable sex ratios were within each year. Student's paired t-tests were used to determine whether sex ratio or density significantly differed between spring and fall. Paired F-tests

were used to determine if the variance in sex ratio or population density differed between these two time periods.

I also used a Pearson's correlation to test for a negative relationship between adult sex ratio and YY sex ratio. This would support Fisher's theory of frequency-dependent selection on sex ratio, where if the population is male biased then selection should favour more females in the next generation.

Masculinity

To test LRC in this system, Pearson's correlations were used to determine if there were associations between sex ratio and population density, masculinity and population density, as well as sex ratio and masculinity.

Variation in masculinity, measured as AGD, was investigated using two separate linear mixed effect models. The first model contained log(body length), the proportion males within each litter, and sex as fixed effects, with litter as a random effect, and was used to determine the effect of these parameters on log(AGD). The second model used the residuals from this mixed effects model plotted against population density, with time period as a random effect, to determine if AGD significantly changed with population density when taking body length, sex ratio, and sex into account.

Seasonal changes in masculinity were investigated using a linear mixed effect model with, body size, sex, percent males, and Julian date as fixed effects, with litter as a random effect. Sex ratio and masculinity has also been associated with maternal care (Moore and Morelli 1985). As a result we investigated the relationship between maternal

care and sex ratio or masculinity using a linear mixed effect models with litter as a random effect.

All statistical analyses were performed in R version 2.13.2 (R Development Core Team 2011) in the nlme package (Pinheiro et al. 2011). All values are presented as mean \pm s.e.m. unless otherwise indicated.

RESULTS

Sex ratio

There was no significant correlation between YY sex ratio and population density across years ($r = -0.36$, $p = 0.17$; Figure 3.2), or during the spring ($r = 0.42$, $p = 0.14$), but there may be an association in the opposite direction from our prediction during the fall ($r = -0.51$, $p = 0.06$). However, I did see a significant negative correlation between YY sex ratio and population density when I de-trend these data ($r = -0.51$, $p = 0.04$). Mean population density significantly differed between spring and fall for each year ($t = -4.27$, $df = 13$, $p < 0.0001$) where densities were on average higher but not more variable ($F_{14,14} = 0.88$, $p = 0.81$) in the fall than in the spring. YY sex ratio did not significantly differ between spring and fall for each year ($t = -1.11$, $df = 13$, $p = 0.29$), but sex ratios during the fall were significantly more variable than sex ratios during the spring ($F_{13,15} = 4.29$, $p = 0.009$). Sex ratio does not appear to be associated with population density in this *Peromyscus* system.

Fisher's theory of frequency dependent selection on sex ratio does not occur within this *Peromyscus* system, as is indicated by a positive correlation between adult sex ratio and YY sex ratio across years ($r = 0.60$, $p = 0.02$).

Masculinity

Between May 12 and August 28, 2011 one hundred and seventy-five *P. maniculatus* offspring were born to 31 dams in a field laboratory setting. The percentage males in each litter varied between 0 and 80 %. Mean population density changed dramatically within this breeding season, where population density rose from 5 captures/100 trap nights in May to 44 captures/100 trap nights in August.

Contrary to the predicted positive correlation between population density and the percent males within a litter, and population density and individual masculinity, the percent males within a litter ($r = -0.31$, $p < 0.0001$), and individual masculinity ($r = -0.36$, $p < 0.0001$) correlate negatively with population density in this system. However, as predicted, masculinity and sex ratios were positively correlated ($r = 0.21$, $p = 0.006$; Figure 3.3).

Using the residuals from a model involving log(AGD) with log(body length), sex ratio, and sex, as fixed effects, regressed against population density with time period as a random effect demonstrated that masculinity declined with population density ($n = 8$, slope = -0.0016 , $p = 0.063$; Figure 3.4b). These results demonstrate that AGD did not increase with population density as was predicted according to LRC.

The proportion of time spent nursing decreased over Julian date, and both the percentage of males within a litter ($n = 31$, slope = 0.54 , $p < 0.001$) and individual AGD

($n = 31$, slope = 0.14, $p = 0.03$) increased with the proportion of time spent nursing. Individual masculinity did not increase over Julian parturition date. This was shown using a linear mixed effect model with Julian date, sex, percentage of each litter that was male, and offspring body size as fixed effects, and litter as a random effect demonstrated that individual masculinity significantly declines over Julian parturition date ($n = 31$, slope = -0.003, $p < 0.001$; Figure 3.5).

DISCUSSION

These results provide little support for the idea that sex ratio is adjusted in response to LRC. Sex ratio (proportion males) and population density were negatively correlated in the long-term data, and a similar trend occurred during the breeding season of 2011, where non-dispersing individuals (females and feminized individuals) tend to be produced during high population density. However, the positive correlation between sex ratio and masculinity supports my prediction that sex ratio and masculinity should occur in the same direction. In other words, dams are adjusting both sex ratio and masculinity but not in a direction that is consistent with LRC within this breeding season.

Support for the LRC hypothesis has been varied (summarized by Sikes 2007). Some possible reasons why LRC may not be supported in some systems are seasonal effects, broad-scale competition rather than local competition, and local mate competition in patchy habitats that are prone to extinction (Sikes 2007). Although other mechanisms of sex ratio manipulation may be present in this population of *Peromyscus* LRC does not seem to be acting. This might be due to inherent seasonality and local mate competition

in patchy habitats. *Peromyscus* experience drastic fluctuations in environmental conditions, food supply, and population densities both within and between seasons (Falls et al. 2007). These fluctuations may override competition for local resources, where at any point the abundance of one ‘resource’ (which could include food, mates, or habitat) may correct for the lack of another resource. Current literature makes a call for the understanding of proximate mechanisms behind sex ratio manipulation (Krackow 2002; Sikes 2007). I add to this proximate understanding by demonstrating that variation in individual masculinity, which reflects testosterone concentrations (Ryan and Vandenberg 2002), does not support sex ratio manipulation in *Peromyscus* in the context of LRC. Completing our understanding of such proximate causes in rodents is essential before we fully understand sex ratio manipulation in these systems.

The results from studies investigating sex ratio manipulation in *Peromyscus* are varied. Wild *Peromyscus* have been shown to support First Cohort Advantage (Goundie and Vessey 1986), Extrinsic Modification (Havelka and Millar 1997; McAdam and Millar 1999), and Trivers-Willard (Goundie and Vessey 1986). Fisher (1930) predicted that population sex ratio and juvenile sex ratio should be negatively correlated, where if the population is male biased then selection should favour more females within the next generation. In these data, there is a positive correlation between adult sex ratio and YY sex ratio, which indicates that frequency dependent selection on sex ratio is not occurring. This positive trend across years may change if investigated on a smaller time scale, where sex ratios may change direction between years and would explain why sex ratio variation does not become fixed on one sex.

I have, however, shown that sex ratio and masculinity are correlated within this *Peromyscus* system. One of the challenges in designing a study associated with masculinity is that there are no clear predictions as to how individual masculinity is associated to behavioural, life-history, or morphological traits in a wild animal. Most of the current work on masculinity has been completed in laboratory rodents (summarized in Ryan and Vandenberg 2002), where there is no mate choice, no differential survival, a potential lack of genetic variance, as well as consistent environmental and social conditions. These studies document the proximate mechanisms behind alterations in individual masculinity, but cannot address ultimate evolutionary questions which requires a natural system where individuals are subjected to fitness tradeoffs and differential survival (Wolff 2003). We need to understand how masculinity varies with ecological variables such as population structure, population density, and environmental fluctuations before we can start to make predictions as to how masculinity is associated to fitness consequences.

Our understanding of the functional and fitness consequences of variation in masculinity is confined due to the limited number of studies that address this variation in wild animals (Cantoni et al. 1999; Monclús and Blumstein 2012). However, we do know that variation in masculinity can have dramatic effects on individual reproductive success, and ultimately, the demography of that species (Monclús and Blumstein 2012). For example, it is generally accepted that intrauterine position has an effect on individual masculinity (Ryan and Vandenberg 2002) and that it has a larger effect on females than it does on males (Cantoni et al. 1999; Ryan and Vandenberg 2002; Monclús and Blumstein 2012). In yellow-bellied marmots (*Marmota flaviventris*), females with short

AGDs are more likely to become pregnant, to wean offspring, and survive for longer periods than masculine females (Monclùs and Blumstein 2012). This pattern is thought to occur in many other mammalian species, where feminized females are faster to enter puberty, have a longer-reproductive lifespan, and are preferred by males (vom Saal and Bronson 1980; vom Saal and Moyer 1985; Drickamer 1996; Zehr et al. 2001). Without understanding individual variation in masculinity we cannot fully understand the affects of sex ratio alterations on the demography and evolution of a population.

In conclusion, this study makes a first attempt at associating masculinity with sex ratio theory and predicts that masculinity should be correlated to sex ratio manipulation in the context of local resource competition. However, in order to apply this novel concept to other theories of sex ratio manipulation and their associated predictions we need to have a greater understanding of how masculinity varies in wild systems. I suggest that more studies should seek to understand manipulation of masculinity in wild systems in general.

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FIGURES

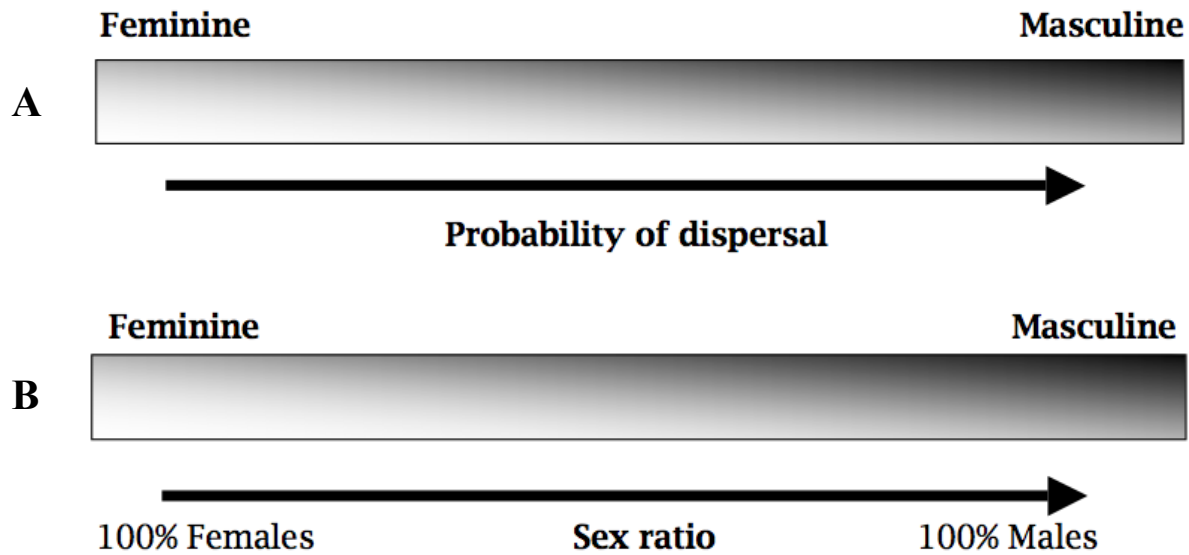


Figure 3.1 - Traditionally, local resource competition predicts that the probability of dispersal can be altered by producing either males or females. If we are to think of this concept in terms of individual masculinity rather than sex, then an individual's masculinity should alter their probability of dispersal (A). However, in the most general form, when integrating population sex ratio and individual masculinity, we predict that masculinity and sex ratio should be correlated, where if producing males increases the dam's fitness then an alternate way of increasing fitness would be to produce masculine individuals (B).

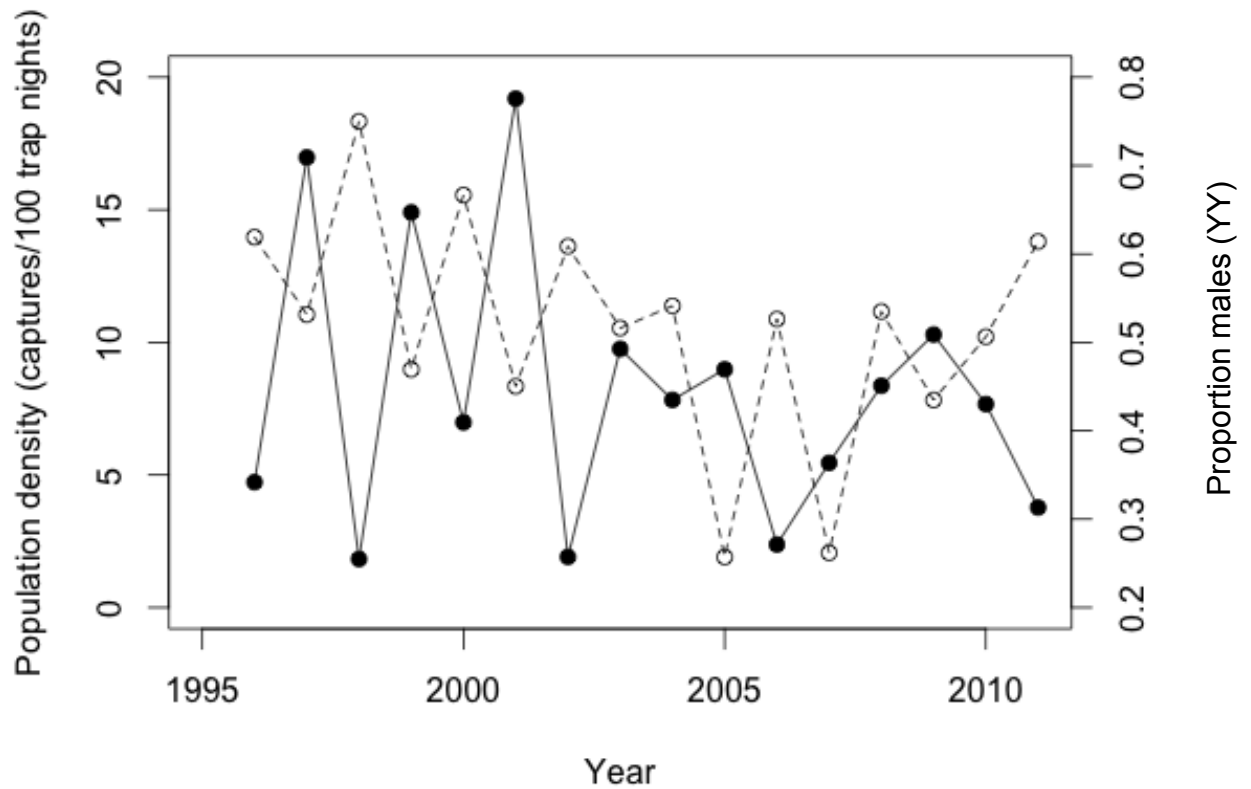


Figure 3.2 - *Peromyscus* population density, including both adult, subadult, and juvenile individuals (dark circles and solid line), and the proportion YY males (open circles with dashed line) vary between years in the long-term Algonquin small mammal survey. When we take year into account by de-trending this data the YY sex ratio and population density are negatively correlated. These data do not support the local resource competition hypothesis as the cause for sex ratio manipulation in this system.

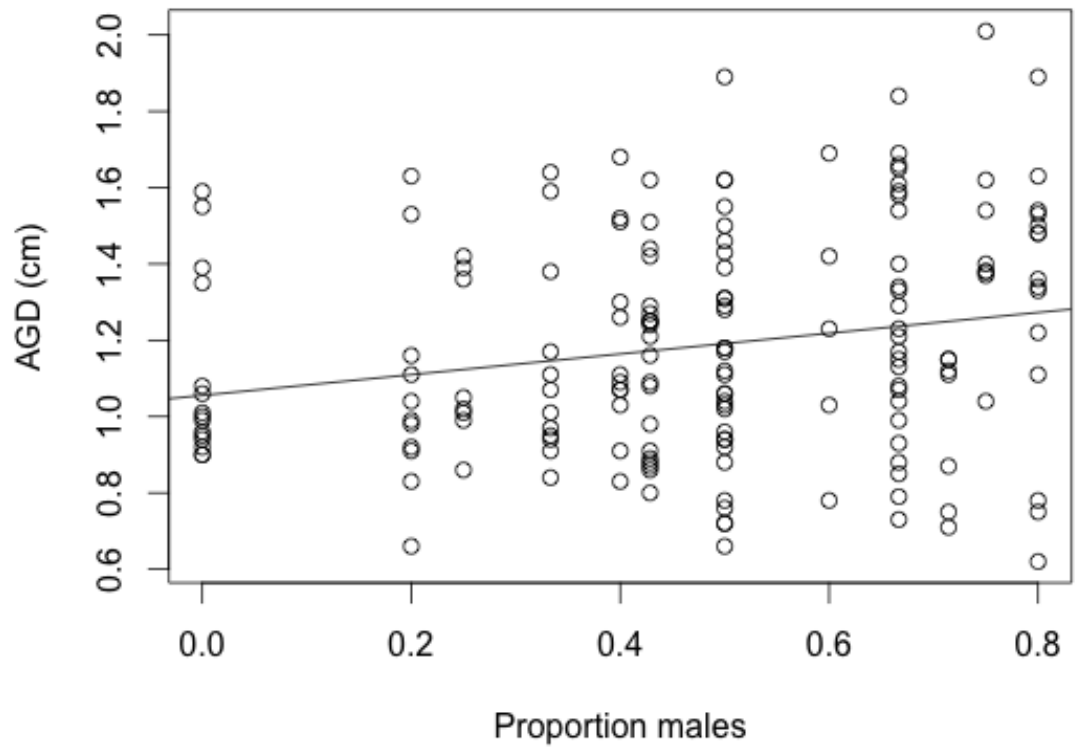
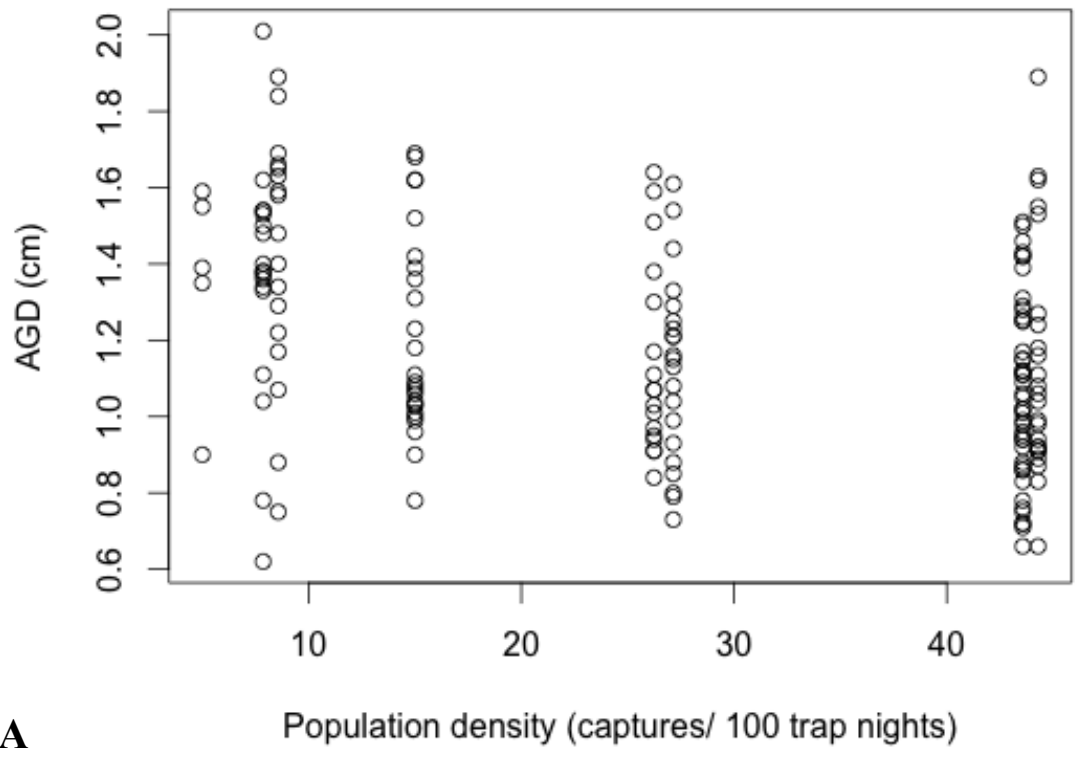


Figure 3.3 - As predicted when using a linear mixed effect model to integrate the concepts of local resource competition and individual masculinity, the sex ratio (proportion males) of each litter was positively correlated to individual masculinity (AGD; cm).



A

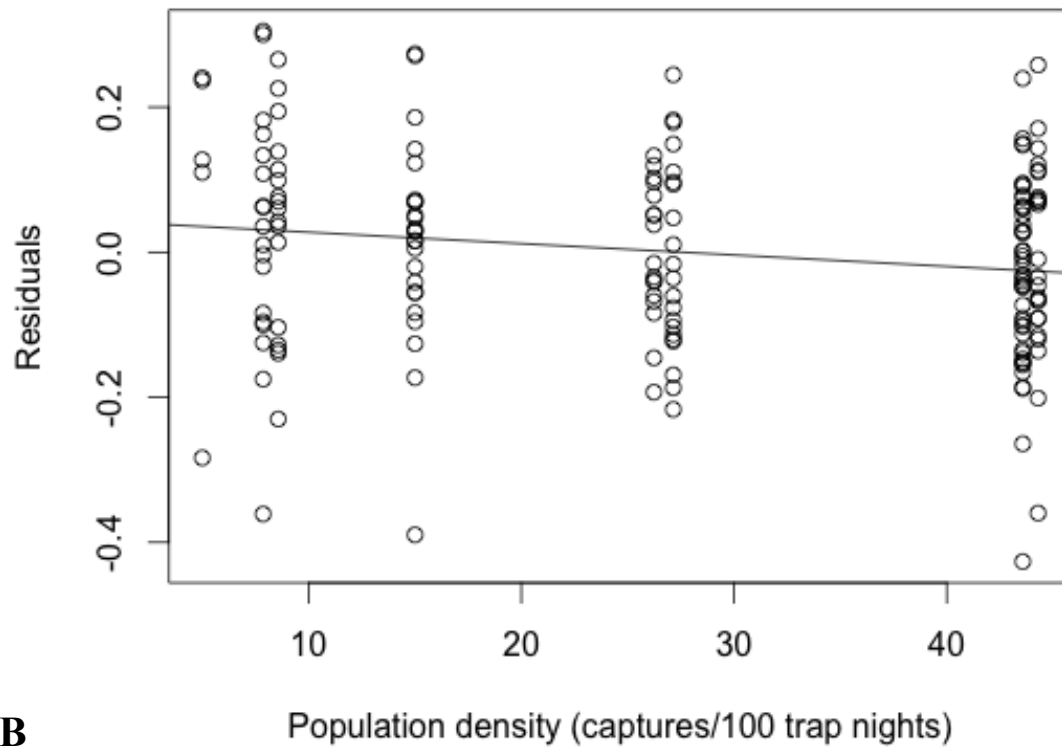


Figure 3.4 - Individual offspring masculinity (AGD; cm), (A) plotted as raw data against population density, and (B) the residuals from a linear mixed effects model that includes sex, percentage male within each litter, and $\log(\text{offspring body length})$, with litter as a random effect, plotted against population density with time period as a random effect. Both graphs demonstrate that AGD does not increase with population density as was predicted. Individuals born during low population density, in May, were more masculine than individuals born during high population density, in August.

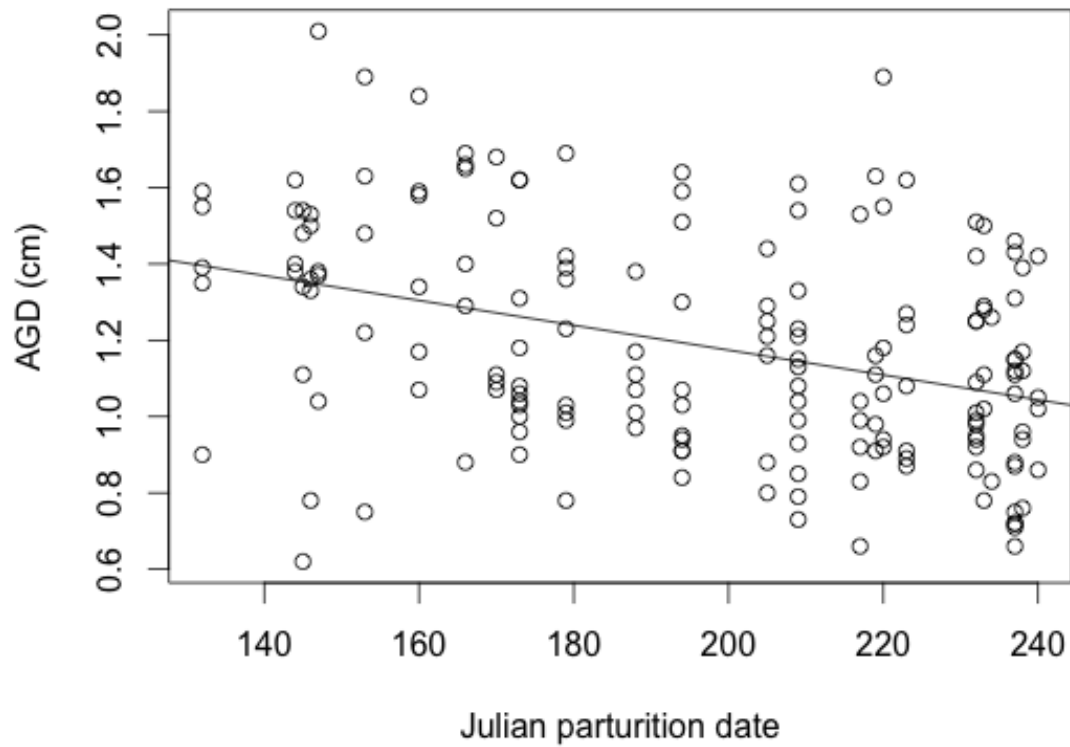


Figure 3.5 – AGD (cm) does not increase over one breeding season when measured with a linear mixed effect model that includes sex, percentage male within each litter, and $\log(\text{offspring body length})$, with litter as a random effect demonstrates that. Individuals born at the beginning of the breeding season, in May, were more masculine than individuals born at the end of the breeding season, in August.

CHAPTER IV

GENERAL CONCLUSION

Maternal care is the physical care given from mother to offspring during a period of postnatal dependency and individual variation in this care has been shown to have important consequences for offspring behaviour, development, and physiology. The proximate mechanisms associated with these maternal effects have been elegantly detailed in laboratory rodents where environmental variation is typically minimized. However, investigating the evolutionary consequences of differences in maternal behaviour and factors that maintain variation in maternal care requires the extension of well established protocols for measuring maternal behaviour in the lab to wild rodent populations. Quantifying maternal care in a wild individual offers the opportunity to integrate laboratory and field studies and to study factors promoting the maintenance of variation in this important trait.

The woodland deer mouse (*Peromyscus maniculatus*) is widespread across North America, abundant, relatively easy to live-trap, and acclimates well to captivity (Kirkland and Layne 1989; Wolff 2003). Because of these traits, they provide an excellent wild model organism to which laboratory-generated hypotheses can be applied. For this reason I chose to study maternal investment in *Peromyscus*, through maternal care and masculinity manipulation, in an attempt test well-established laboratory methodologies in a wild model and to determine whether results from this model were consistent with laboratory findings.

In Chapter II, I quantified individual variation in maternal care using wild *Peromyscus* captured from a population of mice in the Algonquin highlands of central Ontario. I determined that woodland deer mice display the same behaviours as many strains of laboratory mice but that the proportion of time spent nursing declines throughout the natural breeding season. I demonstrated that this decline in proportion of time spent nursing can be accounted for by phenotypic plasticity and that it is tightly correlated to a sharp increase in population density across this same time period.

In Chapter III I tested for a seasonal adjustment of sex ratio and offspring masculinity in *Peromyscus*. I focused on the theory of local resource competition as an intuitive example through which to introduce the novel concept of individual masculinity as a fine-tune adjustment to sex ratio. I determined that local resource competition was not responsible for sex ratio manipulation in this population of *Peromyscus* but I supported my more general prediction that sex ratio and masculinity are correlated and associated with population density.

My results regarding the plasticity of maternal care, and its correlation to population density, are partly consistent with findings from current literature (Champagne et al. 2003a; Champagne et al. 2007; Macrì and Würbel 2006; Macrì et al. 2011). Laboratory literature prominently suggests that maternal care is a fixed phenotype between litters, but that it may vary through lactation and within a night (Meaney 2001; Champagne et al. 2003a; Champagne et al. 2007), however, the extent to this plasticity cannot be investigated in a controlled laboratory setting with no seasonality. More recent research suggests that maternal care may be one form of an adaptive, and plastic, transmission of behaviour across generations (Macrì and Würbel 2006; Danchin et al.

2011; Champagne 2011; Macri et al. 2011), and behavioural plasticity that co-varies with environmental variation implies that the environment might have a profound effect on behavioural phenotype. The behaviour of individuals is often cited as a key component of density-dependent population regulation (Chitty 1967; Krebs 1978, 1996; Sinclair 1989; Mougeot et al. 2003), however, other environmental variables may also be responsible for the observed change in maternal behaviour, such as food availability (Macri and Würbel 2006), and social stability (Kaiser and Sachser 2005; Kemme et al. 2009). I did find evidence of a strong correlation between behavioural plasticity and population density, but because this study mostly involved data from 2011 it is unreplicated across years so I cannot rule out the possibility that maternal care is not plastic in other years. Future studies in this area should firstly investigate whether maternal behaviour is a plastic phenotype in other breeding seasons. Environmental variables, such as population density, will drastically change between years and this study suggests that these changes could have a profound effect on maternal care. Experimental manipulation of variables such as laboratory housing density, temperature, food availability, or litter sex ratio will help to determine which environmental variables account for the observed variation in maternal care.

Although maternal care and sex ratio manipulation appear as two separate concepts within the current literature, there is a subtle suggestion that there may be an association between maternal care and either sex ratio or individual masculinity. In laboratory mice, Moore and Morelli (1979) demonstrate that maternal behaviour is more frequently applied to litters that are male-biased (Moore and Morelli 1979; Baum et al. 1996). Moore (1992) confirmed this observation, and extended it by stating that: “It is

possible that enhanced maternal licking of males has evolved specifically for its contribution to increasing the probability of leaving second-generation offspring through sons". I am unable to comment on Moore's (1992) statement because I did not follow survival and reproduction of juveniles within this study. However, both a decline of in-nest maternal behaviours (Champagne et al. 2003; Weaver et al. 2004; Macrì et al. 2006; Dantzer et al. 2011) and female-biased litters have been associated with maternal stress hormones (Krackow 1995; 1997; Ryan and Vandenberg 2002; Kemme et al. 2009). I demonstrated a correlation between the proportion of time spent nursing, and both the percent of males in a litter and individual masculinity. These variables were associated with an increase in population density, which may have also influenced maternal stress hormones. The associations between maternal care, sex ratio, individual masculinity, and maternal stress make the causal structure difficult to interpret without further investigation within this *Peromyscus* system.

I applied masculinity to sex ratio theory within the context of local resource competition in order to extend this concept to other theories of sex ratio manipulation to gain a greater understanding of the fitness consequences of masculinity in a wild population. Monclùs and Blumstein (2012) have begun this approach in wild yellow-bellied marmots using AGD as a measure of masculinity. They found that masculine females were less likely to survive, engaged in more male-like behaviours, had a lower fecundity and weaning success, and displayed more exploratory-behaviour than feminine females (Laviola et al. 1995; Monclùs et al. 2012; Monclùs and Blumstein 2012). The functional and fitness effects of these associations are difficult to project onto lifetime reproductive success, but one study on wild house mice found that females who are

masculine have an advantage over feminine females during home range size development, which could also influence reproductive success (Zielinski et al. 1992). Cantoni et al. (1999), also found that litter sex composition affected masculinity in female California mice, but not males. This work highlights that variation in masculinity can influence individual fitness, but that these effects might also be sex dependent. Because sex ratio theory addresses the variability of traits between sexes, understanding these associations is crucial when trying to apply masculinity to sex ratio theory. In order to further investigate these questions, a secluded wild population where the fate of individuals can be followed from birth until death is required. This *Peromyscus* system has this capacity, and quantifying individual masculinity in association with individual reproductive success and survival may make for an interesting future study.

In conclusion, I found that maternal behaviour in wild *Peromyscus* is similar to maternal behaviour in laboratory rodents, but that it is a plastic phenotype that is correlated with population density. I concluded that maternal behaviour may change with environmental variation and that future research in this area should attempt to: 1) determine if the direction of plasticity is repeatable between breeding seasons, and 2) narrow down the cause of the observed variation in these wild individuals through experimental manipulation of laboratory housing density density, food availability, or sex ratio manipulation. I also found that local resource competition does not explain the observed sex ratio shift in this population of *Peromyscus*, but that individual offspring masculinity and sex ratio manipulation are positively correlated. Future studies should investigate the fitness consequences of variation in masculinity in wild organisms in

order to better determine whether individual masculinity should be considered when discussing sex ratio manipulation. The next logical steps for developing *Peromyscus* as a wild model for studying maternal behaviour is to first confirm the results of this study by replication over multiple years, and secondly to attempt to understand the transmission of maternal behaviour across generations through cross fostering studies.

This thesis begins to highlight the importance of understanding environmental variation and its influence on behaviour. It demonstrates behavioural plasticity in a wild animal and attempts to draw connections between this phenotype and other life-history traits. It uses an excellent wild model system in which to investigate questions of behavioural ecology and improves our understanding of laboratory results by observing behaviour in a wild rodent.

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