INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

SIBLING RIVALRY FOR MILK IN NORTHERN GRASSHOPPER MICE
(ONYCHOMYS LEUCOGASTER)

A Dissertation

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

Doctor of Philosophy

By

JAMES DONALD MOODIE
Norman, Oklahoma
1999

advisor: Professor Douglas W. Mock, Ph.D.

UMI Number: 9929557

UMI

300 North Zeeb Road
Ann Arbor, MI 48103

SIBLING RIVALRY FOR MILK IN NORTHERN
GRASSHOPPER MICE (ONYCHOMYS
LEUCOGASTER)

A Dissertation APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

Acknowledgments

This dissertation represents the fruits of labor by several people who stayed with me throughout its long gestation. I am grateful to you all.

My desire to work on the behavioral ontogeny of mammals was inspired by my professional relationships with John Byers and Ricky Jannett. Byers gave me the opportunity to study behavior in the field. Ricky enlightened me with his knowledge and skill of small mammal biology in the laboratory. My relationship with these two men forged my wish to study mammalian behavioral development and pursue a Ph.D.

There are several people at the University of Oklahoma who assisted me during the time I spent in Norman. Trish Schwagmeyer always knew of a recent published article that would open my eyes or reinforce my position. Regina Sullivan provided pertinent literature on suckling behavior and encouragement. Walt Kelley and Mark Lomolino invested their time and talents to improve the research and writing. I especially thank Ola Fincke for her questioning, honest approach to it all. Caroline Tawes had all the answers to my office-related questions, always with a smile. Fred Dittmar: you're the man.

There are many graduate students whom I have met, and whose interaction and friendship made my time at Norman not only bearable, but also enjoyable. I can only include a few here. My 'nest-mates' Tim Lamey, Colleen St. Clair, Lynn Haugen, and Jennifer Gieg: the 'sibling' interactions were, for the most part, cooperative rather than competitive. No better office mate can be found than in Colleen. We traded reprints,

books, ideas, and secrets. I thank Lynn for her support when I needed it the most. Thanks to Bob St. Clair for late night discussions of science and life, to Paul Gier, Karen Voltura, Curtis Creighton, Mike Fuller, Greg Smith, Steve Yanoviak, Katie Meier, Stephanie Gurney and too many others to mention for their friendship and assistance.

Several have encouraged me while I was away from Norman. Sharon Dyer insisted I would be better off in the long run to complete the degree. While away from the university, Rosetta Ansotegui consistently goaded me into action when I felt like quitting. The faculty and staff at Idaho State University were understanding and supportive while I wrote and revised.

Though she did not see this project through, I express my gratitude to Michelle. She supported me while I studied for generals, assisted me in the field and laboratory, and shared her passion for life. I thought we had the makings of a great relationship. The adventures we shared were wonderful. Sorry you had such little faith in me, and so little regard for our aspirations.

Doug Mock has gone, in some ways, above his role as advisor. He went beyond the call of duty to pull me out of a dismal situation and encouraged me to complete the degree. Any progress in my professional abilities while attending Oklahoma I owe to Doug. Because of him, I am a better thinker, a better writer, a better scientist. I hope to reimburse him through academic reproductive success.

Finally, I thank my family. Cheers to my parents, Anna and Don, who encouraged me to value education when they had so little themselves. They had no idea that their encouragement would carry me so far. Thanks to my siblings, Vicki, Sue, Tom, Bob, Dan, Paul, Shirley and Marianne, without whom I would not have learned firsthand what it is like growing up within the limits of familial cooperation and competition. And thanks to my second family, Cindy, Sam, Robby, and Boo, who glued me back together when I was broken, believed in me when I did not, and refused to let me fail. This degree belongs to them as much as it does to me. I will never be able to repay you.

TABLE OF CONTENTS

MOTHER'S CONDITION DURING LACTATION INFLUENCES SON'S SEXUAL ATTRACTIVENESS IN GRASSHOPPER MICE

DISSERTATION ABSTRACT

Research on the interactions among close kin has focused primarily on the evolution of cooperative/altruistic behavior. Less appreciated is that the study of interactions between highly related individuals can also provide insight to selfish behavior. Investigation of sibling rivalry offers the opportunity to test the limits of selfish behavior. Though the focus of sibling rivalry research to date has concentrated principally on aggressive resource-based competition, which often leads to the demise of less capable offspring (e.g. egrets, boobies, domestic piglets, hyenas), rivalry can also occur through non-combative behavior such as begging or scrambles for resources. Studies have demonstrated competition through non-aggressive begging in hatchling birds, but little effort has been expended seeking evidence for similar non-combative rivalry in mammals. The research presented here explores non-aggressive sibling rivalry over milk in a small mammal, the northern grasshopper mouse (Onychomys leucogaster). First, I examined the evidence for siblings competing for milk by comparing pup growth and intra-litter variation among different sized litters. Second, by observing suckling behavior, I assessed how mouse pups might compete for milk. Third, I investigated if suckling behavior differed between male and female pups, which might influence the outcome of sibling rivalry. Fourth, I tested female choice for males raised by mothers under good or bad food conditions to determine if early experience can affect subsequent reproductive success.

Food shortages result in sibling rivalry. I examined pup growth and intra-litter variation for 48 lab-reared litters, ranging in size from one to six pups, from the day of birth through weaning. Overall, growth patterns showed a decline in growth rate between days 12 and 18, indicating milk shortages, followed by accelerated growth as pups began ingesting solid food.

Pups from the largest litters grew at slower rates and exhibited greater within-litter variation than did individuals from small litters. Reversals of size ranks among littermates decreased in frequency as pups approached age 18 days. Siblings from larger litters appeared to be competing for milk. Heavier pups were able to maintain their advantage over smaller siblings, but this degree of control diminished for the largest litters (six). Thus, litter size, and quite possibly, the ratio of nipples to pups, influence the intensity of sibling rivalry in grasshopper mice.

How pups can compete for milk is addressed in chapter two. The dilemma faced by a mammalian neonate differs sharply from that of an avian nestling because milk is delivered via multiple simultaneous outlets (nipples), severely constraining a given individual's potential for monopolization. A young mammal's opportunity for acquiring a disproportionate share of its mother's milk seems to be limited to defending nipples that provide greater-than-average quantities of milk or, if quantities are similar across mammary glands, scrambling for milk by sequentially seeking multiple nipples prior to the next milk delivery. This behavior, referred to as 'nipple-switching', may be widespread in taxa that have more functional nipples than concurrent young (a very common pattern across the Mammalia), and that release easily consumed doses of milk in discrete pulses (as opposed to taxa that deliver milk more or less continuously). I sampled the suckling activities of 24 litters of northern grasshopper mice. In this species, small volumes of milk become available during unpredictable let-downs, which occur sporadically (ca 2-7 h^{-1}). To enhance sibling competition further, half of the maternal subjects were maintained on a modestly restricted diet (ca 75% of ad libitum lactating diet). Mean number of nipples acquired per milk let-down was inversely related to litter size. Number of nipples gained per let-down predicted pup growth for individual pups within litters of four, but not in litters of three or five.

Food-restricted mothers sometimes trimmed litter size by killing pups, a practice more frequent and bearing more severe fitness costs for mothers with four pups than for similarly restricted mothers with five pups. The special problems facing pups in litters of four may stem from the non-hierarchical nature of a scramble competition and the number of nipples in this species (six). It is easy to show mathematically that the 4:6 ratio of pups:nipples can generate more intense competition for undrained nipples than 5:6 or 3:6 ratios and therefore the potential for higher intra-litter variance in growth rates.

Preferential treatment of one offspring sex over the other during investment is predicted whenever parents expect a greater return in fitness from the favored sex. Such bias may occur due to gender differences in variance of future reproductive success, early body size dimorphism, or dispersal patterns. I investigated whether northern grasshopper mouse parental investment is skewed to one sex prior to the onset of weaning. I observed the suckling behavior of twenty-four litters ranging in size of three to five pups. Suckling measures included sucking duration following a milk let-down, attempted number of nipple-switches, search duration, and nipple-switching success. I found no indication of gender bias for birth sex ratio, pup mass, or behavioral measures of suckling, except for one trend in which male pups use the hind nipple pair more often than their sisters do. There also was no tendency for mothers to adjust sex ratios in favor of sex via infanticide when faced with limited food resources. In this species, mothers and pups may have little incentive to direct maternal investment towards one sex at the expense of the other.

Female mate choice, which is widely believed to be an important determinant of male reproductive success in some rodents, may be related to body condition of males very early in their lives. I to tested whether sons of northern grasshopper mouse mothers that had

been experimentally food-stressed during lactation were less attractive after reaching sexual maturity than sons whose mothers had had free access to food while nursing their young. I conducted 16 choice tests for male odor using a Y-maze apparatus. Estrous females visited sons of unstressed mothers more often and spent greater amounts of time in chambers associated with such males. Female preference for sons of well-fed mothers suggests that male reproductive success may hinge, at least in part, on adequate maternal investment, which may be influenced by sibling competition.

Overall, the present study provides evidence for early sibling rivalry in a small mammal. The ratio of available nipples to litter size appears to be a key factor in how these rivalries are resolved. For grasshopper mice, nipple-switching behavior is one mechanism that influences which pups gain greater shares of milk over others. Sexual differences in competitive behavior are lacking in this species, suggesting that any sex ratio skews within a litter will not sway outcomes. Finally, it appears that, at least for males, males reared under restricted maternal conditions may be at a disadvantage in terms of future reproductive success, which provides evidence that early experience can affect fitness. This research extends our knowledge of sibling rivalry beyond the few cases found in such peculiar mammalian species such as the domestic pig and spotted hyena. Sibling rivalry may be common in mammalian neonates, and its presence in other species should be investigated. Given the differences in how parental resources are delivered between birds and mammals, we should expect an array of novel strategies in how rivalries are resolved.

LITTER SIZE, GROWTH AND INTRA-LITTER VARIATION: EVIDENCE FOR SIBLING COMPETITION IN THE NORTHERN GRASSHOPPER MOUSE (ONYCHOMYS LEUCOGASTER)

James D. Moodie

Department of Zoology

University of Oklahoma

Norman, OK 73019

Present address:

Department of Biological Sciences

Idaho State University

Pocatello, ID 83209

e-mail: moodjame@isu.edu

fax: 208-236-4570

Abstract

Investigation of sibling rivalry offers the opportunity to test the limits of selfish behavior among close kin. Many studies have demonstrated competition through nonaggressive scrambles in hatchling birds, but little effort has been expended seeking evidence for similar noncombat rivalry in mammals. To determine if grasshopper mouse (Onychomys leucogaster) siblings compete for milk, I measured pup growth and intra-litter variation for 48 lab-reared litters, ranging in size from one to six pups, from the day of birth through weaning (which ends by day 24). Overall, growth patterns reflected a decrease in growth rate between days 12 and 18, indicating milk shortages, followed by accelerated growth as pups start ingesting solid food. Pups from the largest litters grew at slower rates and exhibited greater within-litter variation than did individuals from small litters. Reversals of size ranks among littermates decreased in frequency as pups approached age 18 days. Siblings from larger litters appeared to be competing for milk. Heavier pups were able to maintain their advantage over smaller siblings, but this degree of control diminished for litters of six. I suggest that these results are due to the ratio of pups to nipples and the phenomenon of nipple-shifting for unoccupied nipples as a means of resolving rivalries.

key words: scramble competition, sibling rivalry, litter-size, growth, Onychomys leucogaster

Introduction

Competition among individuals for limited resources is a primary force behind evolutionary change and adaptation (Darwin 1859, Williams 1966). Vital to understanding the outcome of any competitive struggle is determining which aspects of both the resource base and the rivals' abilities to skew resources toward themselves pertain to the conflict. In the context of social interactions among close kin, Hamilton's (1964a, b) seminal papers showed how inclusive fitness automatically modifies competitive costs and benefits, and thus led researchers to focus on identifying the conditions that promote selfish vs. altruistic behaviors. In general, processes affecting non-kin are usually expected to result in self-promoting actions, whereas those impinging on closely related individuals are more likely to promote altruistic traits. There are, however, situations where kin may be in conflict with each other over dangerously scarce resources. This is especially likely where very young offspring remain in close proximity over an extended period and the siblings compete directly for food (Mock and Parker 1997). Sibling competition thus offers an obvious context in which to test the limits of selfish behavior.

For species in which parents provide substantial care, sibling competition typically centers on that commodity (Mock and Parker 1997). Limited, non-shareable forms of care (= parental investment, sensu Trivers 1972), such as food delivered by a parent, can foster conflict among siblings for these resources. The key question, then, is whether an individual should behave altruistically (passing up a resource on behalf of its sibling's welfare) or selfishly (so as to guard itself against future short-falls at its sibling's expense). Resource-based sibling rivalry sometimes produces fatal results (i.e. brood reduction through starvation, siblicide, or

3

infanticide: Mock and Parker 1997), but need not always do so. Short of mortality, sibling rivalry may dramatically affect growing offspring in terms of their potential fitness (Bekoff and Byers 1985). Because of their extensive non-shareable parental investment, both birds and mammals seem to be highly appropriate subjects for exploring sibling competition, yet to date, such work has rarely been performed with mammalian subjects (e.g. Mock et al. 1990, Mock and Parker 1997).

As with other forms of competition, that between siblings can be conveniently classified as one of two types: contests or scrambles (Maynard Smith 1982). Contest competition is often accompanied by aggression in an attempt to exclude rivals from the resource. One variable appearing to promote sibling aggression per se is the extent to which offspring can monopolize provided food (Mock 1985). If items delivered to the nest can be defended economically, an individual may be able to thwart sibling access effectively. A second variable promoting aggression is the possession of neonatal weaponry capable of inflicting injury (Mock et al. 1990). However, most avian sibling competition apparently occurs without overt sibling aggression (Smith and Montgomerie 1990, Lamey and Mock 1991), rather being played out through strategic positioning within the nest and relative strength of begging signals to parents. These scrambles result in chicks vying for insufficient food supplies. Even without aggression, whenever parentally delivered food proves inadequate, the growth of nestmates tends to become increasingly variable relative to that found in broods for which abundant food is available (e.g. Crossner 1977, Magrath 1989, Ohlsson and Smith 1994).

Because most published work on sibling competition has involved birds, it is not surprising that theoretical models are likewise oriented toward avian systems. O'Connor

4

(1978) explored the options of parent and offspring birds as the probability of nestling starvation increases, predicting that sibling rivalry should be more severe in small broods than in larger broods when both are faced with proportionally limited food. This is because the potential gain in food from dispatching a sibling is accordingly greater in smaller broods (i.e. a greater proportion of the hungry mouths are thereby shut). Subsequently, Parker et al. (1989) modeled the payoffs for offspring selfishness vs. nepotism under varying conditions of social hierarchy, brood size, and food restriction. Under a scramble competition scenario (e.g., non-aggressive begging) and assuming that begging has a nontrivial cost (Macnair and Parker 1979, Parker and Macnair 1979, Parker 1985, Harper 1986), a superior player is expected to overwhelm its sib quickly, netting more of the limited resources for Self, even in the face of escalated efforts by its less efficient nestmate. Finally, various models (e.g. Hamilton 1964b, O'Connor 1978, Godfray and Parker 1992) suggest that sibling competition can strongly influence parental fitness, even to the point of favoring filial infanticide. These models predict that if the costs of such competition are shared equally among siblings, an individual within larger broods is likely to be in greater conflict with its sibs. Therefore, the possibility of losing the entire reproductive effort through starvation increases, and the parent can salvage some reproductive success by trimming brood size. Although sibling competition is generally expected to be most severe for smaller brood sizes when faced with a food shortage (O'Connor 1978), the additional marginal benefit of resources gained by a surviving individual after further pruning of the brood may not outweigh the cumulative costs of sibling competition. As well, parents may counter increased competition among brood members by lowering their overall investment in the current brood.

5

In summary, theoretical discussions of avian sibling rivalry have focused attention on three key features: (i) family size (e.g., large vs. small broods); (ii) resource availability (e.g., high vs. low food); and (iii) parental vs. sibling control. Whether these features influence sibling rivalry in similar ways for other taxa featuring substantial parental care has yet to be explored systematically, but such exploration obviously is needed to test the external validity of current avian models.

Although mammals are similar to birds in some respects—family size is small (1-10) and offspring typically require extensive parental care—the two vertebrate classes differ in several relevant attributes. First, for mammalian neonates, only one parent provides most of the care to dependent offspring. Lactation is energetically very expensive (Hanwell and Peaker 1977, Millar 1978, 1979, Galef 1981, Kenagy et al. 1990, Clutton-Brock 1991, Creel and Waser 1991, Sikes 1995), thus likely to be limiting in many cases. Further, though milk supplies increase, mothers seem unable to match elevated demand of larger litter sizes in a linear fashion (Bateman 1957, Edwardson and Eayrs 1967, Fuchs 1982, Leon et. al 1983). Second, food (milk) is delivered to offspring via multiple outlets (nipples). Because average litter size is typically close to one-half the number of nipples, at least in rodents (Gilbert 1984), all offspring within a litter should have access to at least one nipple. At first glance, this method of providing young with nourishment suggests that all siblings have comparable access to food, potentially mitigating sibling competition for milk.

Because littermates in most mammalian taxa are not constrained to a single, common site for food deliveries, one might expect that all offspring should suffer equally if maternal supplies were restricted. There is evidence, however, that other factors—including large litter size and

6

variable quality of a mother's mammary glands—sometimes result in true sibling competition, wherein some individuals grow more rapidly than others, and may even contribute to the demise of lesser littermates. If different mammary glands dispense different amounts of milk, superior siblings may compete for, and actively defend, the better nipples via overt interference competition (e.g., suids: McBride 1963, Fraser 1975, Fraser and Thompson 1991; felids: Ewer 1959, McVittie 1978, Pfeifer 1978, hyraxes: Hoeck 1977, hystricomorph rodents: Kleiman 1972, Gosling et al. 1984). In many other species, where mammary glands may or may not vary in quality, sibling rivalry can take the form of scramble competition for milk. This possibility has been explored in mammals only for domesticated laboratory mice and rats (Bateman 1957, Galler and Turkewitz 1975). For example, Bateman (1957) tested whether birth-weight variation within a rat litter influenced pup growth by fostering two half-litters, four each from lineages selected for large- and small-bodied neonates, into mixed experimental litters. Heavier pups within these mixed litters attained greater masses by age 12 days when competing with "small" littermates than competing against their own "large" sibs in control litters. Bateman concluded that if all pups are similar in size at birth, each suffers constraints on weight gain more or less equally; conversely, if pups vary in initial size, larger sibs obtain more food at the expense of lesser ones, thus grow more rapidly.

Here I explore growth patterns for pups of different litter-sizes that may reveal evidence for sibling competition in northern grasshopper mice (Onychomys leucogaster). Grasshopper mice differ from domesticated mice and rats in having only six nipples and smaller litter sizes (typically 1-6 vs. 8-12 in lab rodents). Specifically, I recorded mean pup masses through time among litters and tested for changes in growth rates across natural variation in litter sizes during the peak period of offspring demand for milk. I also studied within-litter mass variance

over time. Finally, I checked pups for signs of injury that might arise from fighting among littermates. I predicted that, when offspring demand exceeded the mother's ability to provide adequate supplies of milk, larger litters would demonstrate evidence of competition by showing lower mean pup mass than smaller litters. For litters experiencing competition, growth rates should drop especially when pup demand is greatest (just prior to the beginning of weaning). Litters experiencing resource shortages (sib-competition) should also exhibit fewer rank changes in mass and higher within-litter variation of mass than that observed in litters where supply exceeds demand and inter-individual differences in growth are relatively free to be expressed.

Methods

To establish a breeding colony of mice, I collected 52 free-living Onychomys leucogaster in Sherman live traps. Mice were captured in sandsage brush (Artemisia filifolia) habitat in northern Woodward and southern Harper counties in the NW corner of Oklahoma, USA. At the University of Oklahoma's Zoology Department live animal facility in Norman, I housed mice individually in clear plastic cages (29x18x12.5 cm), lined with cedar shavings for bedding and provisioned with ad libitum water and food (rat blocks plus occasional pieces of raw chicken or beef liver). The colony room was maintained at 19 to 23° C with a 16:8 h light-dark cycle.

I randomly assigned adults to heterosexual pairs for mating and subsequently palpated females to detect pregnancies approximately 20 days after their introduction to males. Gestation in this colony was normally 25 to 32 days. For this study, I used data from 54

(subsequently reduced to 48: see below) litters born to 28 females. Though many females (13) contributed more than one litter, the unit of analysis chosen was the individual litter for two reasons: (a) repeat litters tended to be of different sizes (only two of 13 multi-litter females produced the same number of pups and in one of these cases the mother trimmed litter size from five to two within 48 h); and (b) cluster analysis (James and McCulloch 1990) revealed that multiple litters of the same females were no more likely to cluster (with respect to litter size, maternal mass and mean pup birth mass) than unrelated litters. Three litters were excluded from further analysis because of apparent illness of mother or pups. Roughly half of the remaining females determined to be pregnant were separated from their partners (n = 26 litters) and the other half remained with the adult male (n = 25). Of the latter group, most females (n = 16) became pregnant again while simultaneously suckling young. Pregnancy during lactation did not seem to affect litter growth patterns because small rodents are considered generally to be 'income breeders' (Millar 1978, 1979, 1987; Glazier 1985, Perrigo 1987, Thompson 1992, Sikes 1995, 1996)—i.e., mothers rely on the energy supplies currently being ingested—and because litter growth patterns from the two groups of mothers were very similar: mean±1 SD pup mass at 18 days for non-pregnant females = 12.21±2.05 (n = 35) and for pregnant females = 11.93±2.35 (n = 16); Student's t = 0.421, P = 0.677, power for medium effect: 0.496. Consequently, I did not consider this potential variable further. Distribution of initial litter size for these 51 litters (n = 27 females) was (litter size: n): 2:6, 3:13, 4:14, 5:11, 6:5, 7:2 ($\overline{X}$ = 4.04±1.31 SD). Of these litters, 41 remained intact throughout lactation, nine lost one or two pups between zero and two days, and one lost a pup when the litter was between three and five days old. Because partial-litter reductions occurred early, usually within the first day, hence well before important sibling competition is likely to have

played a role, these litters were included in the analyses. Because the largest wild-caught adult female weighed only 52 g (n = 50), I excluded three outlier captive females (mass > 60 g) from further analysis, leaving 48 litters.

I checked pregnant females daily for pups. Upon discovery (day 0), pups were removed from the cage, weighed to the nearest 0.1g using an electronic balance, sexed, and uniquely marked using a 26 gauge needle to tattoo a small amount of India ink under the skin on one or two legs near the junction with the body. The pup was then returned to the natal nest. When pup ears unfolded, typically at 3 d, I uniquely ear-clipped each pup by removing approximately 1 mm^2 of ear (tattoos tended to slough off with flaking skin by 7 d). At the beginning of the project, I removed and weighed mothers on the day of parturition (day 0, n = 41), but signs of maternal infanticide in 17 litters led to a subsequent delay in handling mothers until day three. As a compromise between data resolution and minimization of handling, pups thereafter were measured every third day. Weaning officially ended on day 25 when I placed each litter in a cage separate from the mother where they stayed until reaching 40 days of age, at which time each individual was provided with its own cage.

From individual pup masses, I calculated litter mass, mean pup mass within litters, and mean growth in grams per 3-day intervals. To determine whether maternal mass influenced litter size and pup birth mass, I used linear regression comparing maternal mass (3 d post-partum) with litter size and mean pup birth mass. To estimate the variability of pup mass within litters, I used a relative difference in pup mass (RDPM) index similar to Bryant's (1978) measure for hatchling birds. The index was calculated by taking the difference between the heaviest and lightest pups within a litter, and dividing this by the average mass for those two

10

pups as a scaling procedure. While similar to the coefficient of variation, RDPM places greater emphasis on these two extreme values. I analyzed rank of pups for mass ("mass-rank" = MR) within litters in two ways. First, I counted the number of changes in MR among littermates for consecutive ages; second, I followed the technique of Ohlsson and Smith (1994) by standardizing pup mass to a mean of zero and a variance of one within litters for sizes greater than two and compared these masses to established mass ranks at an earlier age. This method is sensitive to rank changes among extreme ranks (e.g. rising from the lowest rank to highest). Mass data were missing for one cell in each of five different litters (all different ages). For these cells, I estimated mean pup mass by using linear regression of mass and estimated RDPM by using the best fit polynomial regression (R^2 values ranged from 0.42 to 0.99). I did not attempt to estimate MR orders or standardized masses.

To test if initial pup variability influenced the severity of competition, I compared the heaviest pup in the litter at 18 d with its litter's RDPM at birth (day zero) for each litter size. Maximal pup mass was standardized among litters by formulating a maximum pup index for each litter (calculated by dividing the maximum pup mass by its mother's mass). This transformation controlled for the possibility of maternal condition influencing pup growth.

I checked data for departures from normality before applying parametric tests. All t-tests, regressions (including trend analysis to test for nonlinearity of growth curves), ANOVAs and ANCOVAs were performed using SYSTAT software package, version 5.0. For multiple comparison post hoc procedures where sample sizes were unequal, I used the Games-Howell approach, as suggested by Toothaker (1993); for pairwise comparisons within repeated measures, I followed Maxwell and Delaney (1990) employing the modified Tukey's procedure.

11

Results

For 48 litters, maternal mass 3 days postpartum averaged 45.2 g (SD = 5.72). Maternal mass

did not predict mean pup birth mass when all litters were included (n = 48, adj. R^2=0.054,

F=3.66, P=0.06, power=0.81 when testing a medium effect size of 0.16, Sprinthall 1982), or

when only mothers of litters that experienced no mortality were considered (intact litters only:

n = 39, adj. R^2 = 0.034, F = 2.38, P = 0.13, power for medium effect size = 0.72). Maternal

mass did predict litter size (LS) in both samples (all litters: n = 48, adj. R^2 = 0.113, F = 6.98, P

= 0.01; intact litters: n = 39, adj. R^2 = 0.092, F = 4.86, P = 0.03), though accounting for only

about 10% of the variance. Subsequent to partial-litter reduction in 10 litters, LS ranged from

one to seven with a mean of 3.78 and mode of four. Because of a low sample size (n = 2), I

excluded litter sizes of one from further ANOVA and ANCOVA analyses, but note that data

from these singleton litters were consistent with the patterns found in other litter sizes.

Mean pup mass, sorted by age, varied across litter sizes two through six when controlling

for maternal mass (ANCOVA $F_{4, 40}$ = 8.58, P = 0.00004; Table 1). Repeated-measures

ANOVA revealed both a pup age effect ($F_{8, 328}$ = 990.08, P < 0.001) and an age-litter size

interaction ($F_{32, 328}$ = 6.66, P < 0.001), which warranted further analysis of mean pup mass

adjusted for maternal mass (AMPM) across litter size for pup age means and within age

classes.

Visual inspection of adjusted mean pup mass data revealed that litter sizes of five and

six were nearly identical across age (Table 1). Due to low sample sizes of 6-pup litters (n =

5), I compared pup masses within age classes across litter size, pooling litter sizes 5 and 6

(hereafter referred to as LS 5+6), as recommended by Games and Howell (1976) for

samples size < 6 in the Games-Howell multiple comparisons procedure for unequal sample

sizes. For multiple comparisons among overall litter size means, adjusted mean pup mass of

LS 5+6 was always lower than all other litter sizes (family-wise $\alpha = 0.05$). Comparisons of

mean pup masses did not vary across litter sizes two through four. When I compared

AMPMs across litter size within age treatments, by nine days of age pups from LS 5+6 were

lighter than those of all other litter sizes, a pattern that continued through weaning (Table

1). Similarly, by day 18, pups from LS 4 were significantly smaller than those from LS 2.

From birth through 24 days, growth rates for all pups followed a cubic pattern (trend

analysis: $F_{1,45} = 147.13$, P < 0.001, 63.1% of sums of squares [SS] for polynomial contrasts).

Mean pup growth over successive 3 d sampling periods showed an initially low rate (days 0 to

3), then rose in a roughly linear way from days 3 through 12 before slowing temporarily prior

to weaning onset (day 12 to 18), before accelerating again (days 21 through 24: Fig. 1). When

curvilinear trends for growth were analyzed within litter sizes, a linear trend best described LS

2 (linear $F_{1,7} = 30.00$, P = 0.001, 47% of SS), while for all other litter sizes a cubic trend

accounted for ≥ 60% of the variance (LS 3: $F_{1,11} = 66.119$; LS 4: $F_{1,13} = 72.056$; LS 5+6: $F_{1,11} =$

22.53; all P's < 0.001).

Pups in litters of two grew at constant rates while those in larger litters did not (Fig. 2).

Growth varied across litter sizes within the 3 d periods from ages 6-9 d through 21-24 d (Table

2). Post hoc paired t-tests, comparing growth periods 9-12 d and 15-18 d for each litter size

showed that only LS 2 failed to show a decline in growth rate (LS 2: t = 0.59, df = 7, P = 0.58;

13

LS 3: $t = 5.51$, $df = 11$, $P < 0.001$; LS 4: $t = 5.12$, $df = 13$, $P < 0.001$; LS 5+6: $t = 2.69$, $df = 11$, $P = 0.02$). Multiple comparison procedures across litter sizes also revealed that LS 2 growth rates were significantly greater than all other LSs during period 15-18 days (Table 2). Pup growth rate in LS 5+6 fell behind those of LSs 2 and 3 very early (period 6-9 d), while LS 4 was slowed somewhat later (period 12-15 d).

Variability in pup mass within litters rose as litter size increased up to five pups (Fig. 3). A second order polynomial regression fit the data best (RDPM = $-0.0079LS^2 + 0.0842LS - 0.0769$, $R^2 = 0.33$). Using multivariate repeated-measures ANOVA, variation within litters differed across litter size and age, but litter size-age interaction did not (Fig. 4). Variation within litters tended to remain constant across age periods until age 24 days when variation increased.

Within litters the ordering (ranking) of individual pup masses changed frequently between three-day periods for large litters, but remained rather stable over the first 24 days for two-pup litters. However, such rank changes occurred less often before day 18 than thereafter (Table 3). Because LS 2 ranks were consistent, while LS 6 ranks changed repeatedly, I focused on the intermediate litters' rank data. Here (LS 3-LS 5), rank changes occurred less frequently between ages three and 21 days (Table 3), with the fewest changes occurring during the day 12 to 15 period. I also scrutinized the ranks of standardized pup mass for litters of three or more pups at 18 d, when growth rates tended to be minimal based on mass rank at 9 days of age, i.e. prior to declines in growth rates. Although changes in size hierarchy took place initially, high-ranking pups tended to retain their elevated positions (Fig. 5). All of these litter sizes showed

14

strong, negative linear relationships, as first-ranked pups tended to continue to hold that position on day 18, though six-pup litters displayed weaker rank retention than smaller litters.

Correlations between initial pup variability and the largest 18 d old pup's size within a litter were nonsignificant for nearly all litter sizes. Four-pup litters showed a strong, positive relationship between relative difference in pup mass at day zero and the maximum pup mass index (Fig 6).

Discussion

In general, grasshopper mouse mothers produce variable litter sizes of similar-sized neonates, based on a positive association with maternal body condition. Because larger adult females invest in greater numbers of pups and because grasshopper mice are income breeders (Sikes 1995), pup mass might be expected to remain similar across litter sizes throughout the lactation period. This was not the case, despite all mothers having unrestricted access to food. Pups from the largest litters (five and six) fell behind their small-litter counterparts in body mass, as early as six days after birth, and remained in that relative position at least until separated from their mother at 25 days. Similarly, pups from litter sizes of four fell behind those in litters of one or two by day 18 at the latest. These results suggest that even large, well-fed mothers cannot keep up with a large litter's demand for milk, but slip into a situation where pups are resource-limited.

Grasshopper mouse pups typically complete the weaning process by 24 days (Ruffer 1965), despite first beginning to eat solid food shortly after opening their eyes (15 to 18 days old: Sikes

1995, pers. obs.). Some of the observed shifts in Onychomys growth rate may have reflected changes in pup diet. Growth rate was uniformly lowest between 15 and 18 days, then rebounded. Ingestion of rat chow, in addition to any milk, seems likely to have caused this resurgence. As pups consumed solid food, growth may increase rapidly. During the final growth period prior to separation from the mother, mean pup growth rate exceeded the highest growth rate attained while nursing (days 6-9) by 147 percent. Growth rates during the lactation period also differed across litter sizes. Two-pup litters showed a constant rate throughout the lactation period, while growth in larger litters dipped sometime prior to the protracted switch to solid food. Further, as litter size increased, pup growth rate dropped off earlier during the lactation period (Table 2).

These patterns indicate several points. First, above the litter size of two, mothers do not appear to provide sufficient milk to allow pups to grow at a high constant rate, despite an ad lib. diet to mothers. Quantity of milk consumed is tightly correlated with mouse growth as long as pups depend solely on milk (Menz 1980, cited in Fuchs 1982). Second, grasshopper mice exhibited three general growth patterns. Pups from the smallest litters (one, two and three) grew at high and nearly linear rates, whereas those from the largest litters (five and six) grew at lower rates throughout much of lactation. Four-pup litters showed initial growth patterns similar to that of smaller litters, but eventually dropped to rates comparable to pups of larger litters. Because of this combination of high early growth followed by dampened later growth, four-pup litters may experience relatively greater milk shortages during peak lactation time (just prior to the initiation of weaning) than pups of other litter sizes. Third, while pups from the largest litters grew slowly at first, they nevertheless still suffered a subsequent decline in growth rates comparable to that seen in four-pup litters.

16

Evidence that grasshopper mouse pups truly competed during lactation came both from the size disparities among litter-mates over the lactation period and from the persistence of size ranks during the most acute periods of milk shortage. While pups from litters of two seldom displayed rank reversals, such pairs tended to reduce their mass difference as lactation progressed (Fig. 4). The lack of rank reversals by these pups may be because each pup obtained free access to maternal resources: if both individuals added mass at equivalent rates, the relative difference between them would shrink automatically. For litter sizes above two, mass rank reversals occurred frequently. Thus, despite the existence of rank changes, pups occupying the highest or lowest ranks tended to retain them. Additionally, when pup demand apparently outstripped milk supply in larger litters (ca. day 12 to 18), presumably intensifying competition, reversals became less frequent, possibly indicating that higher ranking pups come to maintain their status more tenaciously. During the subsequent transition to solid food (18-24 d), when all pups had free and equal access to rat chow, rank reversals were common.

The relationship between growth rates and relative differences among littermates provides further evidence for sibling competition over milk. Despite growing at suppressed rates, pups in large litters also maintained or increased the relative disparity between heaviest and lightest individuals. In litters of five or six, neonatal variance apparently did not influence growth rates of the largest individuals within litters. There was no correlation between the size of the heaviest pup and the initial litter variance, indicating that early discrepancies did not lead to some pups taking a proportionally greater share of maternal resources. For litters of four, however, the heaviest sib gained relatively more mass if littermate variation was high than if pups were of similar size. These patterns are consistent with Bateman's (1957) experiment

17

using lab mouse litters of eight. Though absolute numbers vary, the ratio of pups to nipples is similar (this study, 4:6; Bateman, 8:12).

There is subtle evidence that six-pup litters, despite facing keen competition, may have less opportunity to resolve intra-litter conflict clearly. Though masses differed within these litters (similar to litters of four and five), variation appeared slightly lower than in five-pup litters (Fig. 3). Ranks changed often among LS 6 littermates, resulting in a less stringent hierarchy, though the lightest pups tended to remain in the inferior positions.

To summarize, this study demonstrated sibling competition for milk by showing: (1) under certain conditions (i.e. increased litter size), food resources did not match offspring demand; (2) pups from smaller litters gained mass more quickly than those from larger ones; (3) individuals from larger litters experienced a decline in growth rate when demand for milk was highest; (4) persistence in mass rank generally occurred when food shortages were likely to be most severe; and (5) relative mass differences among littermates remained constant or increased within larger litters over time but decreased for smaller litter sizes. From this evidence, the key question shifts to how some individuals are able to out-compete siblings during times of resource shortages when each pup has access to a source of nutrition (i.e. one nipple).

Many mammal mothers have discrete milk let-downs throughout a nursing bout with milk being released from numerous mammary alveoli into a common duct that drains into the nipple (reviewed in Grosvenor and Mena 1974, Wakerley et al. 1988). Each of these pulses of milk is available only briefly. Suckling stimulates release of the maternal hormone oxytocin,

18

causing the myoepithelial cells in each alveolus to contract, expelling milk into the duct (Sala and Freire 1974). For many species of mammals, the opportunity to behave selfishly towards one's littermates, when warranted, may arise because an offspring is able to detach from the nipple it drained following let-down, then consume the milk reservoir of a previously unoccupied nipple. Such "nipple-shifting" was first described by Lincoln et al. (1973) for laboratory rats (Rattus). Cramer and Blass (1983) demonstrated that a rat pup gained more milk if it successfully secured a greater number of unoccupied nipples. If some individuals are more likely to shift successfully, they may be able to out-compete their sibs if and when milk becomes limited. Because mass gained is tightly related to milk intake (Menz 1980 cited in Fuchs 1982), pups successful in scrambles for extra milk may maintain or increase neonatal growth differences among pups. With a total of four nipples at stake, a grasshopper mouse in a litter of two should easily be able to make a successful switch during most milk let-downs. As the number of pups increases, the availability of undrained nipples declines, and switching may become less profitable on an absolute scale. This may explain why pups from litter sizes of five and six experienced slower growth rates at earlier ages than those in smaller litters: viz., large-litter pups should have little opportunity to collect extra milk from unoccupied nipples. While mothers might be theoretically able to compensate by nursing pups of larger litters longer or more frequently as pups grow, the collective demand for nourishment in large litters is logically more likely to outstrip the mother's ability to transform solid food into milk than with smaller litters (Bateman 1957, Fuchs 1982, Mendl 1988, Sikes 1995). Of course, if the frequency of nipple-shifting attempts is related to individual physiological needs, very young pups may not bother to shift after every milk let-down because milk supplies may be plentiful for them. This could help explain why alterations in size ranks occurred frequently: pups are not limited by access to milk and by chance, some are gaining more resources than others.

19

However, as demand escalates with growth, larger pups may increase their rate of nipple-shifting or manage to out-scramble rivals for unoccupied nipples, thereby outcompeting their smaller siblings for these available resources.

In addition, some pups within litters may be more skillful and/or persistent than others in finding nipples and maintaining attachment (Gilbert 1995). A pup that cannot secure attachment of any nipple after attempting to shift (either because unoccupied nipples are blocked by littermates or because the pup stops searching) will fail to gain resources during the next milk let-down. Although a few missed let-down opportunities would probably do little to reduce growth during a three-day period (milk yield per mammary gland per let-down in rats is a mere 0.05-0.3 ml: Grosvenor and Mena 1974), a persistent pattern of missed let-downs may cause some pups to fall behind their littermates in growth. The positive relationship between initial variability among littermates and subsequent size of the largest pup in litters of four may indicate that some individuals from these litters have a greater opportunity to out-compete siblings because of the favorable, but uneven ratio of unoccupied-nipples-to-competitors. Behavioral details of grasshopper mouse nipple-shifting are reported elsewhere (Moodie 1999).

Whether size differences among mammalian littermates are sufficient to be biologically relevant remains to be seen. There is evidence that premature weaning of house mice (Mus musculus) increases the probability of death (Fuchs 1981, König and Markl 1987). If mother mice treat the whole litter as a single entity rather than as an assemblage of separate individuals, then any pups lagging in gut development may be unable to digest solid food efficiently once the litter is weaned. These weanlings may not have had sufficient time to make the physiological and morphological gut transitions that are required for switching from a diet of

milk to one of solid food (Henning 1985). Experiments are needed to test variability within litters against the capacity of pups to digest solid food.

While avian sibling competition through aggressive contests can be dramatic and bloody (reviewed in Mock et al. 1990), rivalries resolved through nonaggressive scrambles appear to be more common (e.g., Crossner 1977, Rydén and Bengtsson 1980, Bengtsson and Rydén 1983, Magrath 1989, Smith and Montgomerie 1991, Ohlsson and Smith 1994; see reviews in Lamey and Mock 1991, Mock and Parker 1997). Similarly, there are a growing number of studies for mammalian species where youngsters compete for resources employing weapons (teeth) (hyenas: Frank et. al 1991, pigs: Fraser 1975, Fraser and Thompson 1991, Galápagos fur seals: Trillmich cited in Mock and Parker 1997). Apparently, the use of weapons to combat siblings is not limited to postpartum opportunities, but may happen in utero as well (pronghorns: O'Gara 1969). There appears to be few instances where mammals have even been examined directly for evidence of scramble competition by sibs, and those studies have involved domesticated mice and rats (Bateman 1957, Galler and Turkewitz 1975). In this study I infer sibling competition from measures of growth effects when litter size increases beyond two. To gain a fuller appreciation of the circumstances that intensify sibling rivalries, additional studies on a wide range of species and under field conditions are needed. Because of the high expense of lactation, milk shortages are likely to be widespread. Though studying competition among mammalian siblings will require considerable effort and ingenuity to overcome their secretive nature, these endeavors may illuminate the prevalence of competition for milk and identify which factors most influence how rivalries are settled.

21

Acknowledgments

References

Bateman N (1957) Some physiological aspects of lactation in mice. J Agric Sci 49:60-77

Bekoff M, Byers JA (1985) The development of behavior from evolutionary and ecological perspectives in mammals and birds. In: Hecht MK, Wallace B, Prance GT (eds) Evolutionary Biology, vol 19. Plenum Press, New York, pp 215-286

Bengtsson H, Rydén O (1983) Parental feeding rate in relation to begging behavior in asynchronously hatched broods of the great tit Parus major. Behav Ecol Sociobiol 12:243-251

Bryant DM (1978) Establishment of weight hierarchies in the broods of house martins Delichon urbica. Ibis 120:16-26

Clutton-Brock T (1991) The evolution of parental care. Princeton University Press, Princeton, New Jersey

Cramer CP, Blass EM (1983) Mechanisms of control of milk intake in suckling rats. Am J Physiol 245:R154-159

Creel SR, Waser PM (1991) Failure of reproductive suppression in dwarf mongoose (Helogale parvula): accident or adaptation? Behav Ecol 2:7-16

Crossner KA (1977) Natural selection and clutch size in the European starling. Ecology 58:885-892

Darwin C (1859) On the origin of species by means of natural selection. John Murray Press, London

Edwardson JA, Eayrs JT (1967) Neural factors in the maintenance of lactation in the rat. J Endocr 38:51-59

Ewer RF (1959) Suckling behaviour in kittens. Behaviour 15:146-162

Frank LG, Glickman SE, Licht P (1991) Fatal sibling aggression, precocial development, and androgens in neonatal spotted hyaenas. Science 252:702-704

Fraser D (1975) The "teat order" of suckling pigs. II. Fighting during suckling and the effects of clipping the eye teeth. J Agric Sci 84:393-399

Fraser D, Thompson BK (1991) Armed sibling rivalry among suckling piglets. Behav Ecol Sociobiol 29:9-15

Fuchs S (1981) Consequences of premature weaning on the reproduction of mothers and offspring in laboratory mice. Z Tierpsychol 55:19-32

Fuchs S (1982) Optimality of parental investment: the influence of nursing on reproductive success of mother and female young house mice. Behav Ecol Sociobiol 10:39-51

Galef BG Jr (1981) The ecology of weaning. In: Gubernick DJ, Klopfer PH (eds) Paternal care in mammals. Plenum Press, New York, pp 211-241

Galler JA, Turkewitz G (1975) Variability of the effects of rearing in a large litter on the development of the rat. Develop Psychobiol 8:325-331

Games PA, Howell JF (1976) Pairwise multiple comparison procedures with unequal $\underline{n}$'s and/or variances. J Ed Stat 1:113-125

Gilbert AN (1984) Mammary number and litter size in Rodentia: the "one-half rule". Proc Natl Acad Sci 83:4828-4830

Gilbert AN (1995) Tenacious nipple attachment in rodents: the sibling competition hypothesis. Anim Behav 50:881-891

Gittleman JL, Thompson SD (1988) Energy allocation in mammalian reproduction. Am Zool 28:863-875

Glazier DS (1985) Energetics of litter size in five species of Peromyscus with generalizations for other mammals. J Mamm 66:629-642

Godfray HCJ, Parker GA (1992) Sibling competition, parent-offspring conflict and clutch size. Anim Behav 43:473-490

Gosling LM, Baker SJ, Wright KMH (1984) Differential investment by female coypus (Myocaster coypus) during lactation. Symp Zoo Soc Lond 51:273-300

Grosvenor CE, Mena F (1974) Neural and hormonal control of milk secretion and milk ejection. In: Larson BL, Smith VR (eds) Lactation: a comprehensive treatise. Academic Press, New York, 227-276

Hamilton WD (1964a) The genetical evolution of social behaviour. I J Theor Biol 7:1-16

Hamilton WD (1964b) The genetical evolution of social behaviour. II. J Theor Biol 7:17-52

Hanwell A, Peaker M (1977) Physiological effects of lactation on the mother. Symp Zool Soc Lond 41:297-312

Harper A (1986) The evolution of begging: sibling competition and parent-offspring conflict. Am Nat 128:99-114

Henning SJ (1985) Ontogeny of enzymes in the small intestine. Ann Rev Physiol 47:231-245

Hoeck HN (1977) "Teat order" in Hyrax (Procavia johnstoni and Heterohyrax brucei). Z Säugetierk 42:112-115

James FC, McCulloch CE (1990) Multivariate analysis in ecology and systematics: panacea or pandora's box?. Ann Rev Ecol Syst 21:129-166

Kenagy GJ (1987) Energy allocation for reproduction in the golden-mantled ground squirrel. Symp zool Soc Lond 57:259-273

Kenagy GJ, Masman D, Sharbaugh SM, Nagy KA (1990) Energy expenditure during lactation in relation to litter size in free-living golden-mantled ground squirrels. J Anim Ecol 59:73-88

Kleiman D (1972) Maternal behaviour of the green acouchi (Myoprocta pratti Pocock), a South American caviomorph rodent. Behaviour 43:48-84

König B, Markl H (1987) Maternal care in house mice. I. The weaning strategy as a means of parental manipulation of offspring quality. Behav Ecol Sociobiol 20:1-9

König B, Riester J, Markl H (1988) Maternal care in house mice (Mus musculus): II. The energy cost of lactation as a function of litter size. J Zool Lond 216:195-210

Lamey TC, Mock DW (1991) Nonaggressive brood reduction in birds. Acta XX Congr Int Ornith, Wellington, New Zealand, pp 1741-1751

Leon M, Fischette C, Chee P, Woodside B (1983) Energetic limits on reproduction: interaction of thermal and dietary factors. Physiol Behav 30:937-943

Lincoln DW, Hill A, Wakerley JB (1973) The milk-ejection reflex of the rat an intermittent function not abolished by surgical levels of anaesthesia. J Endocr 57:459-476

McBride G (1963) The "teat order" and communication in young pigs. Anim Behav 11:53-56

McVittie R (1978) Nursing behavior of snow leopard cubs. Appl Anim Ethol 4:159-168

Magrath RD (1989) Hatching asynchrony and reproductive success in the blackbird. Nature 339:536-538

Macnair MR, Parker GA (1979) Models of parent-offspring conflict. III. Intra-brood conflict. Anim Behav 27:1202-1209

Maynard Smith J (1982) Evolution and the Theory of Games. Cambridge University Press, Cambridge

Maxwell SE, Delaney HD (1990) Designing Experiments and Analyzing Data: a model comparison perspective. Brooks/Cole Publishing, Pacific Grove, CA

Mendl M (1988) The effects of litter size variation on mother-offspring relationships and behavioural and physical development in several mammalian species (principally rodents). J Zool Lond 215:15-34

Millar JS (1978) Energetics of reproduction in Peromyscus leucopus: the cost of lactation. Ecology 59: 1055-1061

Millar JS (1979) Energetics of lactation in Peromyscus maniculatus. Canadian J Zool 57:1015-1019

Millar JS (1987) Energy reserves in breeding small rodents. Symp Zool Soc Lond 57:231-241

Mock DW (1985) Siblicidal brood reduction: the prey-size hypothesis. Am Nat 125:327-343

Mock DW, Drummond H, Stinson CH (1990) Avian siblicide. Am Sci 78:438-449

Mock DW, Parker GA (1997) Evolution of Sibling Rivalry. Oxford Press, Oxford

Moodie JD (1999) Sibling rivalry for milk in northern grasshopper mice (Onychomys leucogaster). Unpublished dissertation, University of Oklahoma, Norman, OK

O'Connor RJ (1978) Brood reduction in birds: selection for fratricide, infanticide and suicide? Anim Behav 26:79-96

O'Gara B (1969) Unique aspects of reproduction in the female pronghorn (Antilocapra americana). Amer J Anat 125:217-232

Ohlsson T, Smith HG (1994) Development and maintenance of nestling size hierarchies in the European starling. Wilson Bull 106:448-455

Parker GA (1985) Models of parent-offspring conflict. V. Effects of the behavior of the two parents. Anim Behav 33:519-533

Parker GA, Macnair MR (1979) Models of parent-offspring conflict. IV. Suppression: evolutionary retaliation of the parent. Anim Behav 27:1210-1235

Parker GA, Mock DW, Lamey TC (1989) How selfish should stronger sibs be? Am Nat 133:846-868

Perrigo G (1987) Breeding and feeding strategies in deer mice and house mice when females are challenged to work for their food. Anim Behav 35:1298-1316

Pfeifer S (1980) Role of the nursing order in social development of mountain lion kittens. Develop Psychobiol 13:47-53

Ruffer DG (1965) Sexual behaviour of the northern grasshopper mouse (Onychomys leucogaster). Anim Behav 13:447-452

Rydén O, Bengtsson G (1980) Differential begging and locomotory behaviour by early and late hatched nestlings affecting the distribution of food in asynchronously hatched broods of altricial birds. Z Tierpsychol 53:209-224

Sala NL, Freire F (1974) Relationship between ultrastructure and response to oxytocin of the mammary myoepithelium throughout pregnancy and lactation: effect of estrogen and progesterone. Biol Reprod. 11:7-17

Sikes RS (1995) Costs of lactation and optimal litter size in northern grasshopper mice (Onychomys leucogaster). J Mamm 76:348-357

Sikes RS (1996) Tactics of maternal investment of northern grasshopper mice in response to postnatal restrictions of food. J Mamm 77:1092-1101

Smith HG, Montgomerie R (1991) Nestling American robins compete with siblings by begging. Behav Ecol Sociobiol 29:307-312

Sprinthall RC (1982) Basic Statistical Analysis. Addison-Wesley, Reading, MA

Stearns, SC (1992) The evolution of life histories. Oxford University Press, New York

Thompson SD (1992) Gestation and lactation in small mammals: basal metabolic rate and the limits of energy use. In: Tomasi TE, Horton TH (eds) Mammalian Energetics: interdisciplinary views of metabolism and reproduction. Cornell University Press, Ithaca, NY, pp 213-259

Toothaker LE (1993) Multiple comparison procedures. Sage University Paper series on Quantitative Applications in the Social Sciences, 07-089. Sage, Newbury Park, CA

Trivers RL (1972) Parental investment and sexual selection. In: B Campbell (ed) Sexual Selection and the Descent of Man, Aldine, Chicago, pp 136-179

Wakerley JB, Clarke G, Summerlee AJS (1988) Milk ejection and its control. In: Knobil E, Neill J (eds) Physiology of Reproduction, Raven Press, New York, pp 2283-2321

Williams GC (1966) Adaptation and natural selection Princeton University Press, Princeton, New Jersey

Table 1. Mean pup mass (adjusted for maternal mass) and one standard deviation for 48 litters by litter size across age. Pups not surviving first five days postpartum not included in calculation of means.

	Litter Size										
	1		2		3		4		5		6	
n	2		8		12		14		7		5	
Age (days)	X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
0	2.73	0.27	3.26	0.51	3.37	0.31	3.24	0.27	3.08	0.25	3.05	0.50
3	4.65	1.06	4.70	0.50	4.61	0.71	4.38	0.53	3.99	0.50	3.90	0.68
6	7.18	0.36	6.57	0.84	6.47	1.07	6.15	0.95	5.30	0.87	5.28	1.12
9	9.75	0.52	8.56	0.96	8.48	1.45	7.90	1.32	6.58	1.12	6.58	1.42
12	12.43	0.99	10.42	0.84	10.24	1.63	9.49	1.46	7.83	1.18	7.95	1.53
15	15.01	1.98	12.34	0.81	11.87	1.82	10.69	1.47	8.87	1.26	9.02	1.69
18	16.94	0.99	14.04	0.74	13.13	2.06	11.70	1.54	9.87	1.37	9.90	1.40
21	18.77	0.21	16.04	1.25	14.78	2.68	13.35	2.08	10.56	1.53	10.97	2.05

(Table I continued)

24	21.17	1.27	19.24	1.52	17.41	3.03	16.14	2.53	12.37	2.74	13.30	2.94
X	12.07	0.85	10.57	0.89	10.04	1.64	9.23	1.35	7.61	1.20	7.77	1.48

Table 2. ANCOVA analyses (F values) and probabilities (p) for growth rates by litter size within growth periods (in days of age). Lines connecting litter sizes represent non-significant post-hoc Games-Howell pairwise multiple comparison procedures using a family-wise $p = 0.05$. All other pairwise comparisons differed significantly.

Growth Period (days)	$F_{3,41}$	P	Litter Size 2	3	4	5+6
0 to 3	2.20	0.10	——	——	——	——
3 to 6	3.35	0.03	——	——	——	——
6 to 9	8.18	0.0002	——	——	——	
9 to 12	6.50	0.001	——	——	——	——
12 to 15	15.32	<0.0005	——	——		——
15 to 18	9.61	<0.0005		——	——	——
18 to 21	5.52	0.003		——	——	——
21 to 24	3.09	0.04	——	——	——	——

Table 3. Mass rank changes within litters by litter size from the beginning to end of age period. N = no changes in rank among all pups within a litter, C = at least one change in rank among pups. Examination for the directionality of rank changes vs. no changes made using sign tests. Sample sizes within litter size class may differ across age periods due to missing values for that time period.

| Period | Rank | Litter Size | | | | | All Litters | | | Litters 3, 4, and 5 | | |
		2	3	4	5	6	Total	Sign Test	P	Total	Sign Test	P
0-3	N	7	10	5	5	1	28	-1.64	0.10	20	-1.22	0.22
	C	0	2	9	2	4	17			13		
3-6	N	7	9	10	5	2	33	-2.94	0.003	24	-2.61	0.009
	C	1	3	4	2	3	13			9		
6-9	N	7	10	9	6	3	35	-3.54	0.0004	25	-2.96	0.003
	C	1	2	5	1	2	11			8		
9-12	N	7	10	8	5	1	31	-2.53	0.01	23	-2.47	0.13
	C	1	2	5	2	4	14			9		
12-15	N	8	11	12	5	2	38	-4.42	0.00001	28	-4.00	0.00006
	C	0	1	2	2	3	8			5		

32

(Table III continued)

15-18	N	7	10	10	4	1	32	-2.83	0.004	24	-2.83	0.004
	C	1	2	4	2	4	13			8		
18-21	N	6	9	7	2	0	24	-0.29	0.77	15	-0.52	0.60
	C	2	3	7	5	5	22			17		
21-24	N	7	7	6	4	0	24	-0.29	0.77	17	-0.17	0.86
	C	1	5	8	3	5	22			16		

Figure Legends

Figure 1. Mean growth of pups over three day periods, for all litter sizes from birth to age 24 days. Bars represent 95% confidence intervals. Matching letters indicate groups that do not differ significantly according to a modified Tukey's pairwise comparison procedure for repeated measures using error rate familywise $\alpha=0.05$.

Figure 2. Mean pup growth as a function of litter size, adjusted for maternal mass and measured across three-day intervals. Bars represent 95% confidence intervals. Pairwise multiple comparison procedures employed the Games-Howell statistic for unequal sample sizes. Error rate familywise $\alpha=0.05$.

a=LS 5+6 significantly different from LS's 2, 3 and 4: all other pairwise comparisons did not differ.

b=LS 5+6 significantly different from LS's 2 and 3: all other pairwise comparisons did not differ.

c=LS 4 and LS 5+6 significantly different from LS 2 and LS 3: all other pairwise comparisons did not differ.

d=LS 2 significantly different from LS's 3, 4 and 5+6: all other pairwise comparisons did not differ.

e=LS 5+6 significantly different from LS 2: all other pairwise comparisons did not differ.


Figure 3. Overall relative difference of pup mass (RDPM) for litter sizes two through six. Data fitted with polynomial regression curve (RDPM=-0.0079LS^2 + 0.084LS - 0.077, R^2=0.33, F=9.92, P=0.0003). Dashed lines are 95% confidence intervals.

Figure 4. Relative difference of pup mass (RDPM) across age by litter size, for litters two through six. Multivariate repeated-measures ANOVA for litter size: $F_{4,39}=5.05$, $P=0.002$; for age: $F_{8,32}=2.34$, $P=0.04$; and for litter size-age interaction: $F_{32,122}=1.12$, $P=0.31$.

Figure 5. Relationship of the mass rank held when 9 days old and mean standardized pup mass at age 18 days ($\pm95\%$ CI). '1' represents highest rank (heaviest pup in a litter). Litter mass was standardized to a mean of zero and variance of one within litters. Letters represent panels of different litter size (a=LS 3: $R^2=0.65$, $F_{1,9}=59.8$, $P=0.00003$; b=LS 4: $R^2=0.429$, $F_{1,12}=42.3$ $P=0.00003$, c=LS 5: $R^2=0.642$, $F_{1,4}=53.2$, $P=0.002$; d=LS 6: $R^2=0.441$, $F_{1,3}=23.8$, $P=0.02$).
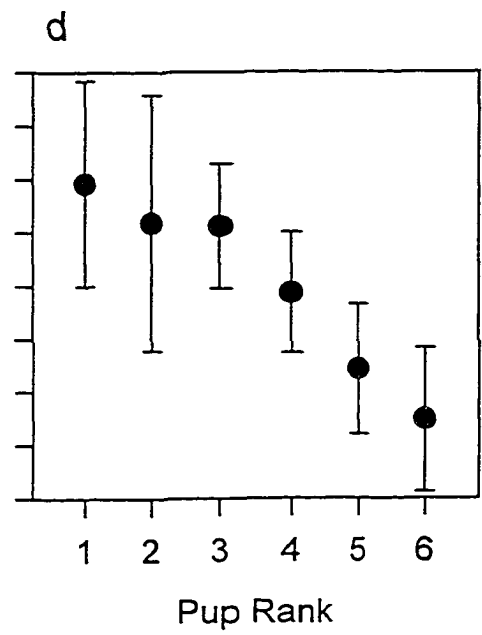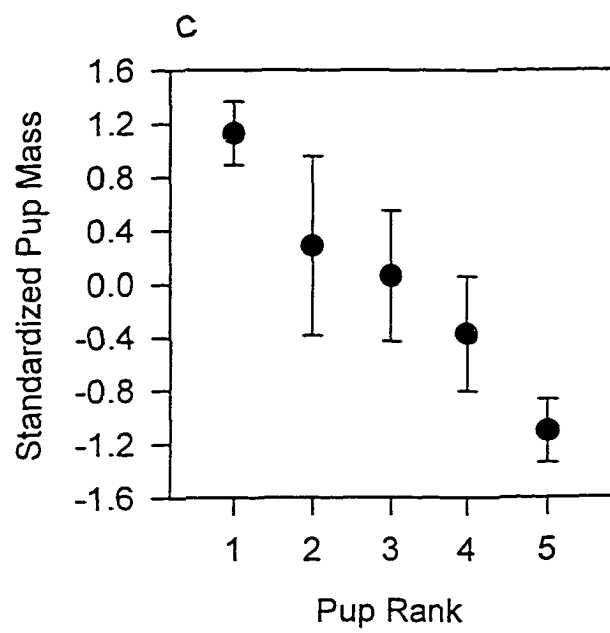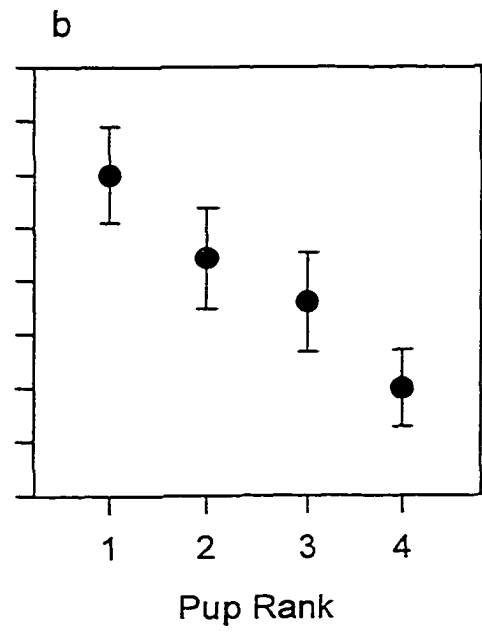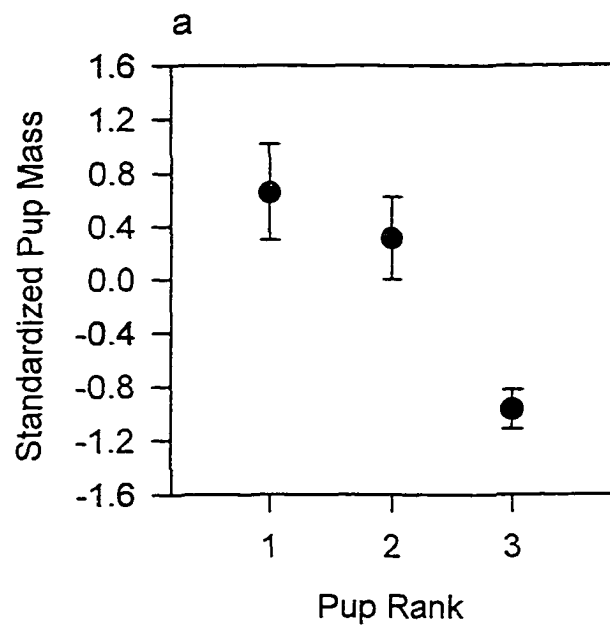
Figure 6. Relationship between initial relative difference of pup mass (RDPM at day zero) and the index of maximum pup mass (largest pup's mass divided by mother's mass) within a litter at day 18 (Pearson's $r=0.607$, $n=14$, $P=0.021$).
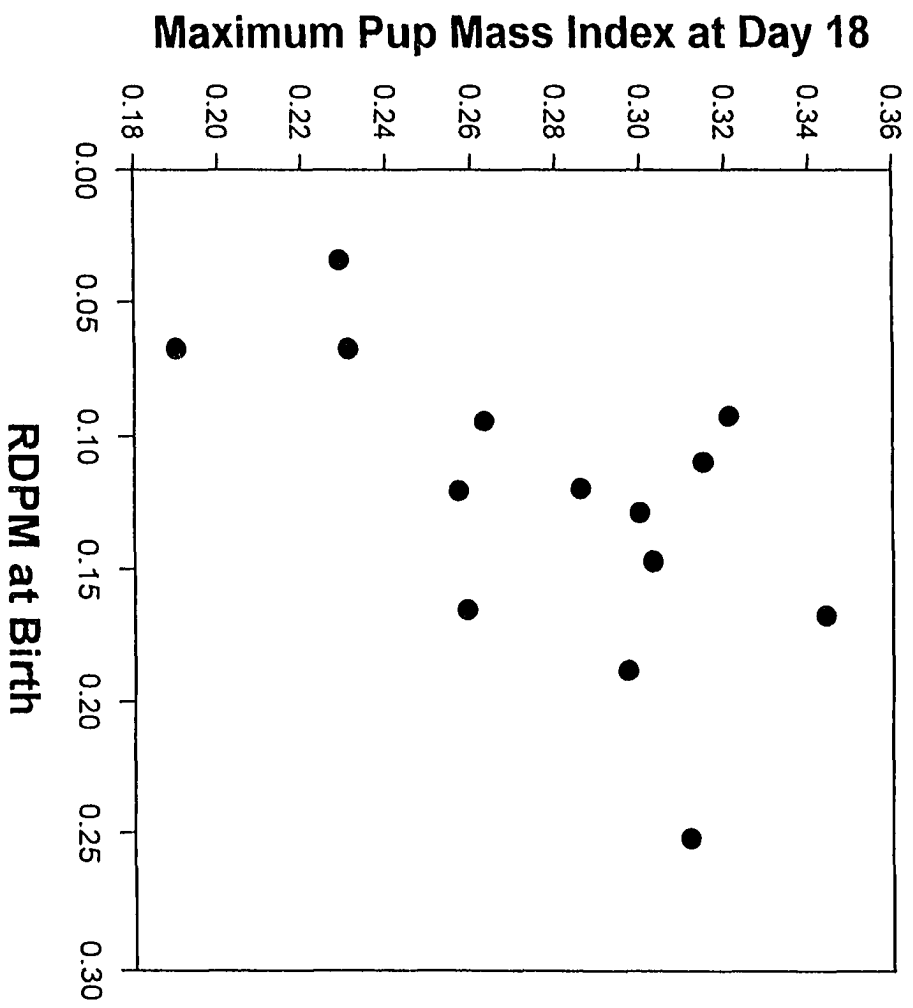
Growth (g)

Age (days)

37
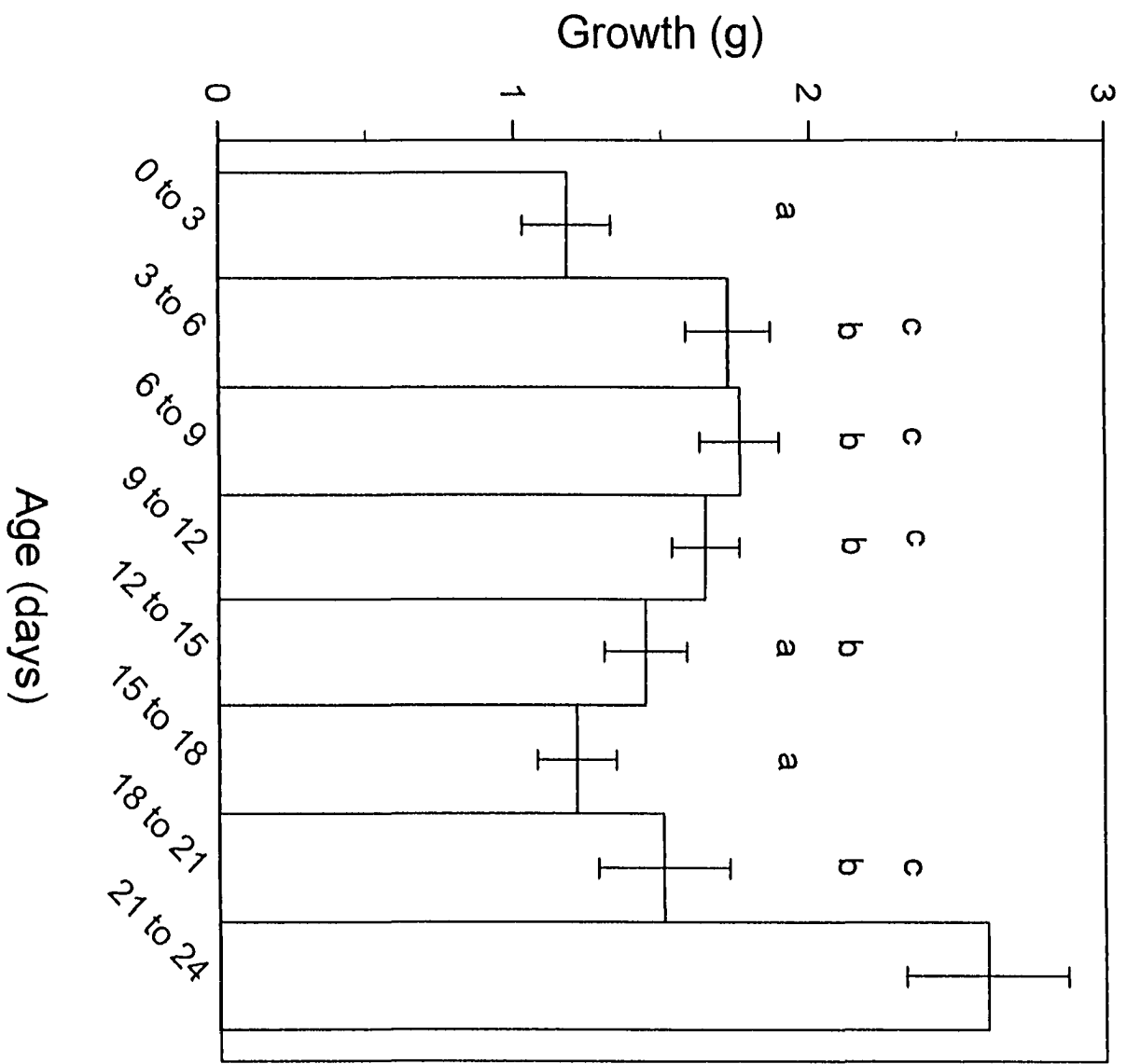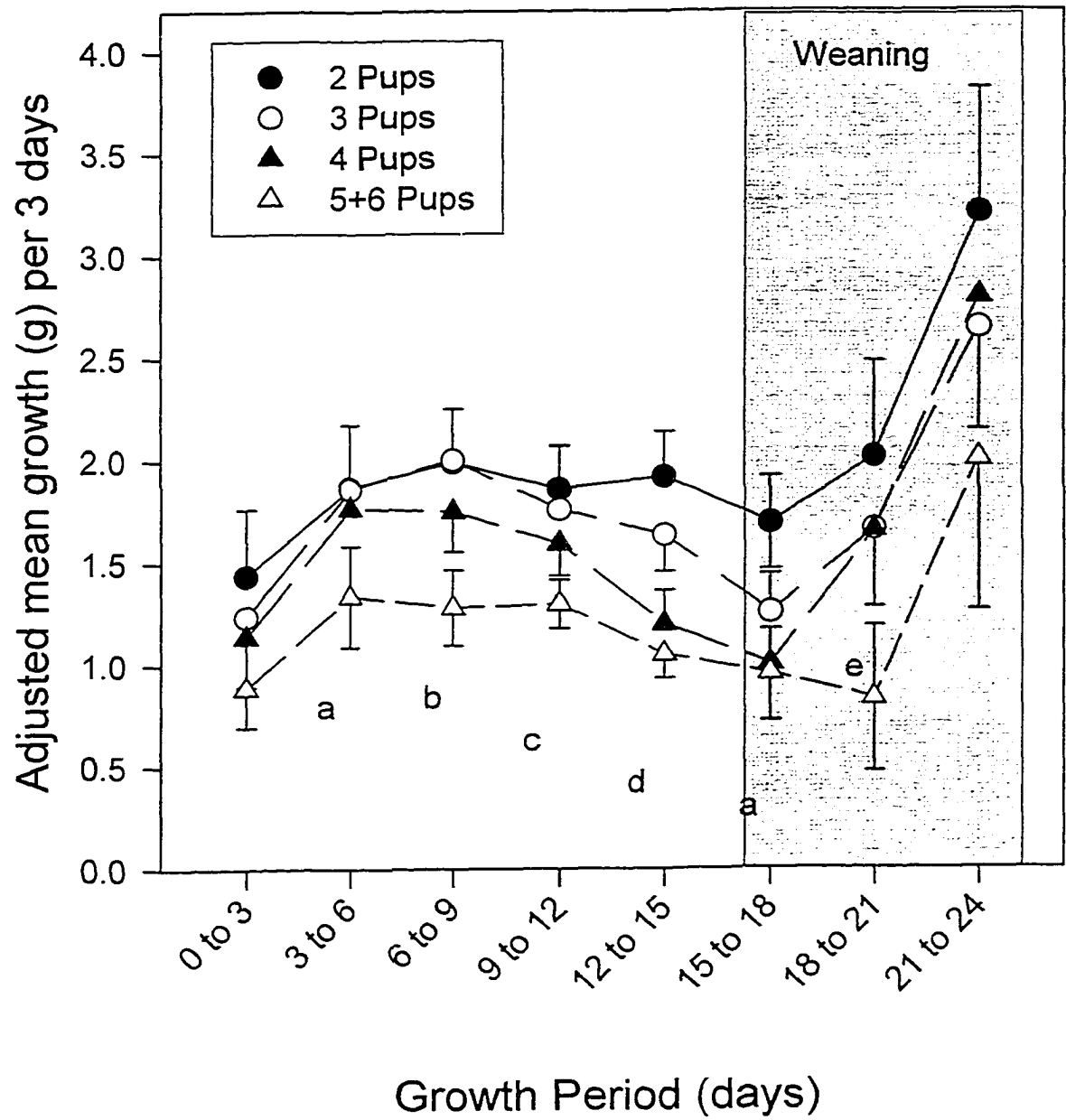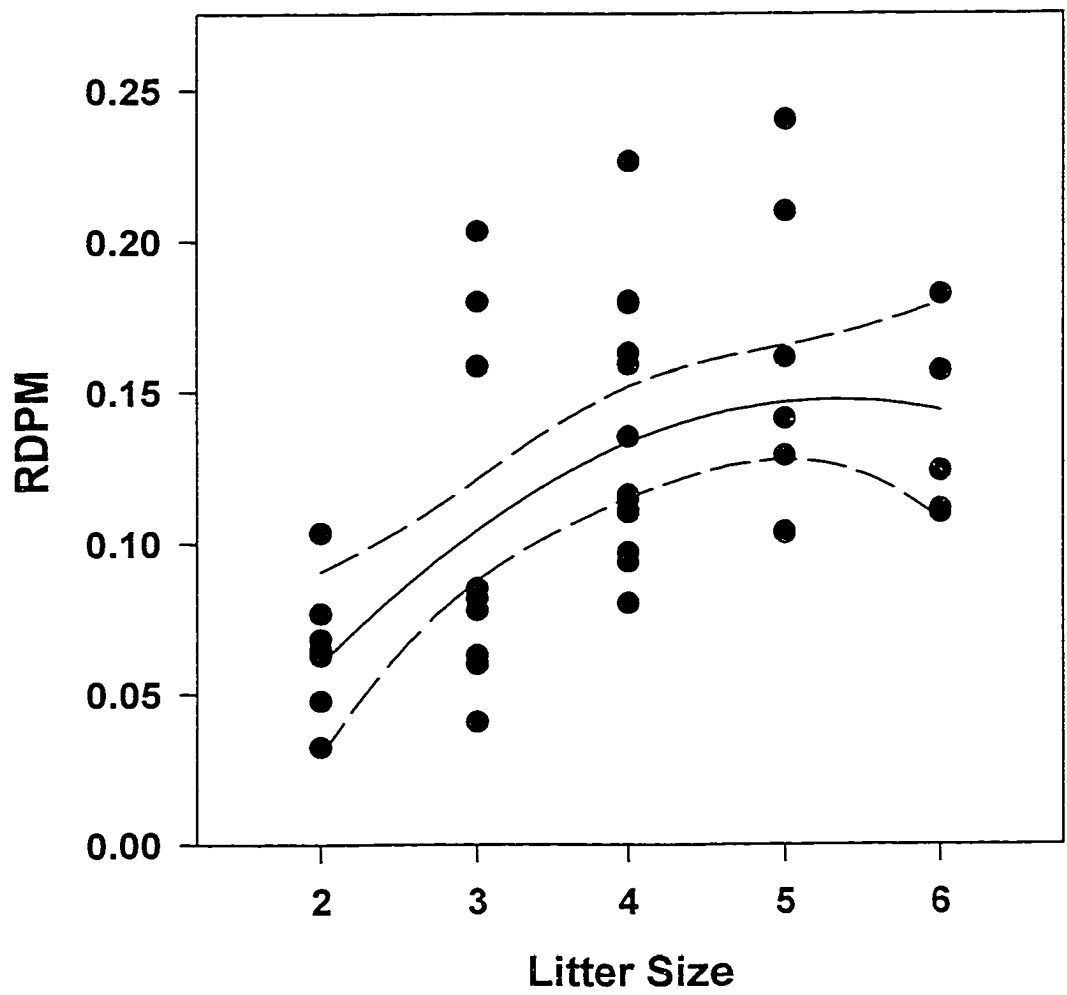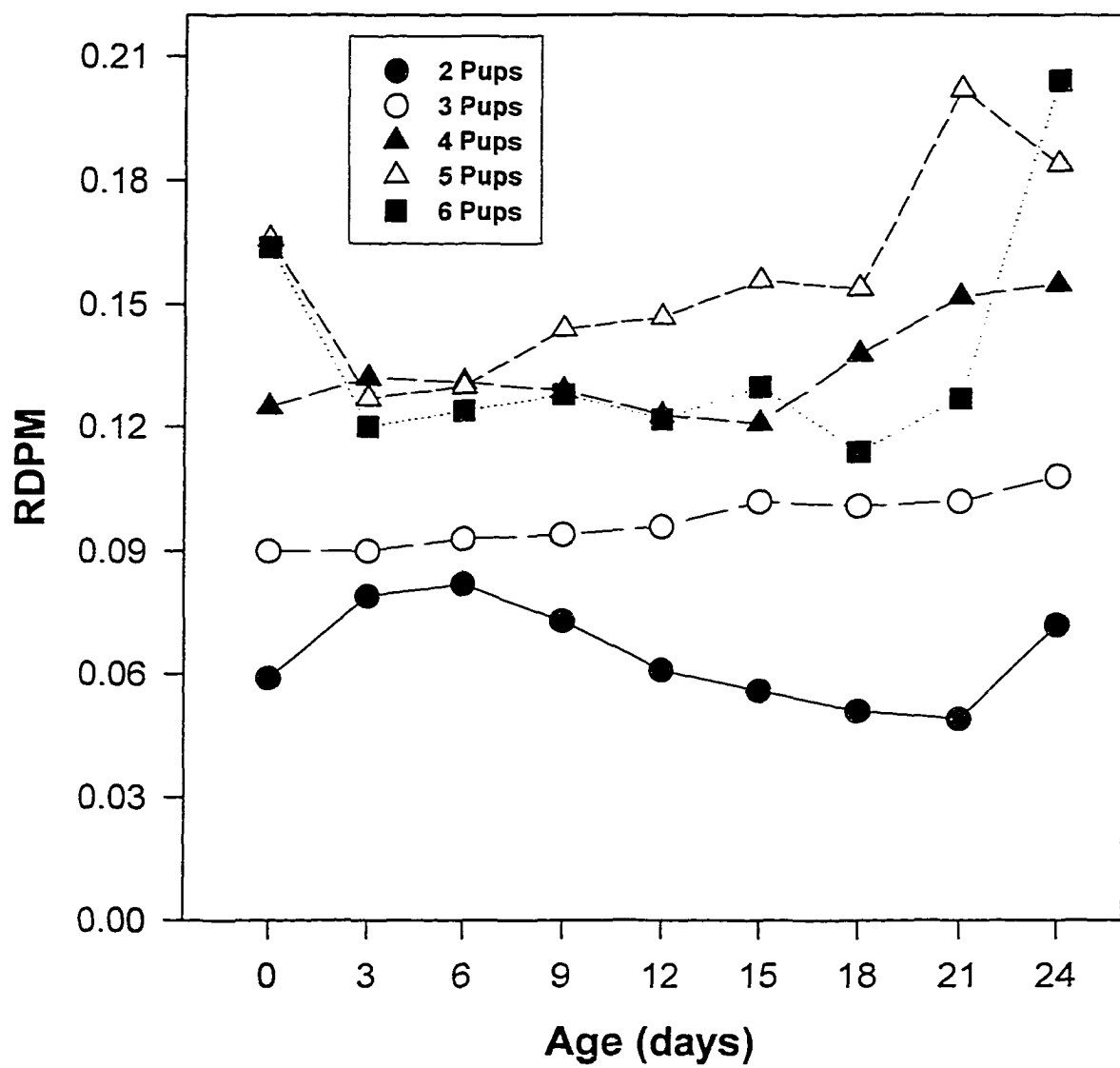
THE ROLE OF NIPPLE-SWITCHING IN GRASSHOPPER MOUSE SIBLING RIVALRY

Moodie: sibling rivalry for milk

JAMES D. MOODIE

Department of Zoology, University of Oklahoma

Norman, Oklahoma 73019 USA

Correspondence:

Department of Biological Sciences

Idaho State University

Pocatello, ID 83209-8007 USA

e-mail: moodjame@isu.edu

Abstract

In sexual species, even the closest of genetic kin come into conflict when shared key resources are in short supply. But the dilemma faced by a mammalian neonate in such circumstances differs sharply from that of an avian nestling because milk is delivered via multiple outlets (nipples), severely constraining a given individual's potential for monopolization. A young mammal's opportunity for acquiring a disproportionate share of its mother's milk seems either to be limited to fatal aggression or to a type of scramble competition called 'nipple-switching'. This latter behavior may be widespread in taxa that have more functional nipples than concurrent young and that release easily consumed doses of milk in discrete pulses (as opposed to taxa that store larger milk volumes, delivering fewer very large meals). Nipple-switching consists simply of a neonate draining the small volume available in the nipple to which it was originally attached as soon as a milk is released event occurs and quickly moving to a second undrained nipple to collect a second dose. I sampled the suckling activities of 24 litters of northern grasshopper mice, Onychomys leucogaster. In this species, small volumes of milk become available during unpredictable let-downs, which occur sporadically (ca 2-7 h^{-1}). To enhance the sibling competition, half of the maternal subjects were maintained on a modestly restricted diet (ca 75% of ad libitum lactating diet). Mean number of nipples acquired per milk let-down was inversely related to litter size. Number of nipples gained per let-down predicted relative pup growth for individual pups within litters of four, but not in litters of three or five. Food-restricted mothers sometimes trimmed litter size by killing pups, a practice more frequent and more severe for mothers with four pups than similarly restricted mothers with five pups. The special problems facing pups in litters of four may stem from the non-hierarchical nature of a scramble competition and the number of nipples in this species

43

(six): it is easy to show mathematically that the 4:6 ratio of pups to nipples can generate more intense competition for undrained nipples than a 5:6 ratio and therefore the potential for higher intra-litter variance in growth rates. The ratio of mammalian litter size to nipple number is likely to be an important factor influencing the expression of sibling rivalry in many mammals.

Introduction

Hamilton's (1964) rule defined the conditions under which individuals should act altruistically toward others as a function of genetic relatedness. His simple inequality (br-c >0, where b = net benefit to the recipient of the act, r = coefficient of relatedness between performer and recipient, and c = net cost to the performer) has generated an extensive literature documenting nepotism in a variety of species. The flip side of the rule, defining when individuals should behave selfishly towards kin, is less commonly explored. Sibling rivalry should be expected when close kin are confined to a small area and where demand for shared resources may outstrip supply (Mock & Parker 1997, 1998). For litters with more than one offspring and provided with extensive parental care, considerable potential exists for acute sibling rivalry.

While sibling rivalry has now been studied closely in quite a few avian species (reviewed in Mock & Parker 1997), mammalian sibling rivalry remains relatively unexplored (for two key exceptions see Fraser & Thompson 1991, Frank et al. 1991). Mammals, like birds, show extensive parental care (though the burden normally falls exclusively on the mother); young stay confined to a nest or burrow for an extended period, and litter sizes are often greater than one. Because the litter sizes of small mammals commonly vary within- and between-species, and because the mother's current food resources can have immediate impact on her offspring in the so-called 'income-breeding' species (Sibly & Calow 1986, Sikes 1995a, 1996a, Moses et al. 1998), these taxa offer a largely untapped resource for the comparative study of sibling rivalry. Unlike birds, however, parental nutrients are delivered to neonatal young simultaneously via multiple outlets, the mother's nipples. Because of the delivery system architecture, the potential for any individual offspring to monopolize parental investment may

45

be somewhat limited. Additionally, for many rodents, the mean number of offspring is approximately half the number of the mother's nipples (Gilbert 1984) and the provision of two nipples per dependent young should further mitigate against inequalities in resource consumption. Litter sizes half as large as the number of nipples may also buffer young against short-term food shortages (Moodie 1999), while larger litters suffer greater mortality and/or reduced growth (Sikes 1995a, b, 1996a, b, Moses et al. 1998). Offspring of many small mammals rely on mothers accessing a constant, adequate food supply (income breeders) because these species are unable to store energy for reproduction (capital breeders) as can larger species such as red deer (Cervus elaphus; Clutton-Brock 1991, Stearns 1992).

Nevertheless, rat (Rattus rattus), house mouse (Mus musculus), and grasshopper mouse (Onychomys leucogaster) littermates can vary considerably in mass, developmental rate, and survival, suggesting that pups may indeed compete for sustenance (Bateman 1957, Fuchs 1982, Moodie 1999). Further, small differences in body size, at least for males, can affect reproductive success (Krackow 1993). A possible strategy for gaining greater proportions of milk was suggested by Cramer & Blass (1983): in some rodents, a milk let-down (MLD: the oxytocin-induced expressing of milk from the alveolar tissue of the mammary) occurs as a discrete event. Immediately following a let-down, each pup quickly drains the nipple to which it is attached, an action easily recognizable in most species by the exaggerated outstretching by the pup's body. If the number of functional nipples exceeds that of consumers, a speedy individual can sometimes release its first nipple and attempt to attach to an undrained one, thereby gaining extra milk. In a study of laboratory rats, Cramer & Blass (1983) found that the relative amount of milk consumed by each pup during a nursing bout matched the number of unused nipples to which that pup switched. If some young are able to gain extra milk more

46

consistently than their littermates, nipple-switching may allow pups to out-scramble siblings for maternal resources.

To investigate whether nipple-switching is used by non-domesticated mammals for competing with siblings, I designed an experiment to test the effects of nipple-switching on pup growth and survival. Because free-living rodents are unlikely to have infinite supplies of food for the mother to use in re-stocking her milk supplies, I compared the behavior and growth effects of litters whose mothers were mildly food-restricted, while also examining natural variation in litter size.

Methods

For this study, I established a breeding colony of northern grasshopper mice at the University of Oklahoma's Zoology Department live animal facility. Grasshopper mouse females possess six mammae, and commonly give birth to litters ranging in size of two to six (McCarty 1978). Wild mice were captured in Harper Co., OK. From that stock, a sample of healthy females (weighing between 38 and 48 g at time of mating) was allowed to mate with males chosen at random. When each female was determined to be pregnant, she was removed to a nursery cage (42.5 x 22 x 13 cm), and supplied with a nesting box plus bedding material. For the experiment, I used 24 litters, each from a different mother, in a 2 x 3 factorial design for food availability and litter size, with repeated measures on litter age. I recorded natural litter size (3, 4, or 5 pups). Litters above or below this range were excluded. Each litter was randomly assigned to a food availability treatment. For litters designated as ad libitum feeders, the mother had free access to food (= rat chow blocks) throughout her lactation. I weighed food every three days to determine consumption. Mothers of litters assigned to the food-restricted

diet received approximately 75% (based on previous unpublished data) of the mean consumed by the ad libitum mothers from day 0 until day (d) 18. Mothers did not differ in mass among the treatment groups for litter size ($\overline{X} \pm SD$ = 3-pup: 43.96 ± 3.97; 4-pup: 43.90 ± 3.52; 5-pup: 43.16 ± 3.84, N's = 8) and diet (Ad libitum: 44.78 ± 3.73; Ration: 42.57 ± 3.32; N's = 12) on day 3 of pup age. Because pups usually begin ingesting solid food around age 18 d (pers. obs.), I restored the restricted mothers to a full ad libitum diet on this day.

Upon discovering neonates, I weighed each pup to the nearest 0.1 g on an electronic balance, then sexed and uniquely marked it using a 26 gauge hypodermic needle to inject a small tattoo of India ink under the skin on one or two legs near the junction with the body. When pup ears unfolded, typically on day 3, I uniquely ear-clipped each pup by removing approximately 1 mm^2 of ear (tattoos tended to slough off with flaking skin by 7 d). To facilitate individual recognition during suckling bouts, I marked pups without adequate hair with a white paint pen. Each pup received a unique pattern of paint marks on the head and belly. After pups had sufficient fur (approximately 9 days of age), I hair-dyed pups on their dorsal sides with unique patterns of Lady Clairol Platinum Blonde hair dye.

To reduce the risk of infanticide due to handling, I did not begin weighing mothers until day three post partum, and pups and mothers thereafter were measured every third day. I ended weaning on day 25, placing each litter in a cage separate from the mother.

To facilitate observations of the nursing process, on day six I transferred the mother to a clean, clear plastic cage devoid of bedding materials and supplied with a opaque, cylindrical

plastic nest-box, 11 cm in diameter. The nest-box was covered with a ceramic tile square (11x11 cm), and a clear Plexiglas floor (13x13 cm). A 4 cm diameter hole in the side allowed access to and from the nest box. Following an hour-long separation from their mother, I introduced the pups to this new 'observation' nest-box. The cage and nest-box were then elevated above an angled mirror and observed from behind a screen so that I could see the mother's ventral surface clearly at close range (90 cm) without being seen. When the mother stopped moving sufficiently to allow suckling, I recorded the behavioural events, either directly or via videotape for 1-2 h. I repeated this procedure every three days through day 15 (four samples per litter). If no milk let-downs were recorded during an observation period, I returned the mother and her litter to the observation cage the following days and observed or taped a second or third session. In all, I recorded 682 MLDs over 98 observation periods ($\overline{X}$ ± SD: 4.51 ± 2.00 MLDs per hour).

Though I used a much less restrictive restricted diet compared with other rodent food-limitation studies (McClure 1981, Sikes 1995b, 1996a, Moses et al. 1998), six of 12 restricted litters experienced reduction in pup numbers (versus 0 of 12 ad lib. litters). To minimize pup deaths during the experiment, I used only four litters per treatment cell (litter size x food availability). Though it was not possible to watch all pups continuously, I also observed five cases in which food-unrestricted mothers (females not included in this study) fatally bit their own pups; Sikes (1995a, 1996a) also has reported active infanticide by female grasshopper mice. In addition, maternal infanticide is known to occur in other rodents, where it may serve an adaptive litter-trimming role (e.g., Day & Galef 1977). The five observed victims were killed almost instantaneously from a single bite.

49

I used parametric statistical tests when appropriate (Sokal & Rolf 1995). When data violated assumptions, I employed nonparametric tests (Siegel & Castellan 1988). I used JMP software (1996 SAS institute) to conduct all tests.

Results

For the first three days post-partum, mothers of the two food treatments did not differ significantly in mass, but thereafter mothers on restricted diets weighed less than those on ad libitum diets. This pattern persisted until free access to food was restored (fig. 1). Mothers on restricted diets consistently lost mass from 3 d ($\overline{X} \pm SD = 42.56 \pm 3.32$ g) to 12 d (38.88 ± 2.58 g; paired-$t_{11} = 4.87$, $P < 0.001$), when maternal mass stabilized. Following restoration of full diet, the formerly restricted female mass quickly rebounded to eclipse 3 d levels (24 d: 45.23 ± 2.46 g; paired-$t_{11} = -2.89$, $P = 0.01$).

During the first 18 days, litter size and food availability to mothers affected pup growth rates with larger litters and pups of restricted mothers growing more slowly. Pup growth rates were equivalent across treatments thereafter (fig. 2). Growth rates increased for all litters older than 18 d, independent of treatment group (mean difference $\pm$ 95% confidence intervals: 0.31 ± 0.07 g, paired-$t_{23} = 8.27$, $P < 0.001$), perhaps due to food restricted mothers being transferred to an ad libitum diet at this time.

Overall, mothers averaged 4.58 ± 1.23 SD milk let-downs (MLDs) per hour, not differing across litter size (MANOVA: $F_{2, 17} = 0.26$, $P = 0.77$) or between maternal diet treatments ($F_{1, 17}$

$= 0.27, \underline{P} = 0.61$). There was a trend, however, for MLD rate to increase as pups aged from day 6 through day 15 (repeated measures MANOVA: $\underline{F}_{3, 15} = 2.80, \underline{P} = 0.075$). The interval between consecutive MLDs averaged 11.90 min (n = 95 observation periods with at least two MLDs) and varied widely (range: 1.65-68.78 minutes). Sequential MLDs appeared unpredictable. Using autocorrelation analysis, the subsequent MLD interval was unrelated to its predecessor when considering all MLD intervals (n = 492, $\underline{r} = 0.05, \underline{P} = 0.25$) or when considering each female separately (Table I).

The mean number of nipples gained by a pup per MLD was negatively related to litter size (fig. 3), but was not demonstrably related to maternal diet (MANOVA: $\underline{F}_{1, 12} = 2.69, \underline{P} = 0.13$). For all intact litters, mean pup growth rate over the 12-15 d period was related to mean nipples gained per pup per hour of observation during 12-14 d suckling bouts ($\underline{R}^2 = 0.35, \underline{F}_{1, 16} = 8.56, \underline{P} = 0.01$), but was not evident at other ages (Table II). Success at gaining greater numbers of nipples for the prevailing pup was a strong predictor of its daily growth rate through 18 d in four-pup litters (fig. 4). Nevertheless, this effect could not be demonstrated for litters of three- (Wilcoxon signed rank test $\underline{T} = 0, N = 8, \underline{P} = 0.50$) or five-pups ($\underline{T} = -1.5$, N = 5, $\underline{P} = 0.75$).

The within litter mass variation, represented by relative difference of pup mass (RDPM = {maximum pup-mass $-$ minimum pup-mass}/ mean pup-mass), was more variable among 4-pup litters ($s^2 = 0.0048$) than among 5-pup litters ($s^2 = 0.0007$) at 15 days ($\underline{F}_{4, 4} = 6.86, \underline{P} = 0.04$). Four- and 5-pup litters are similar in the variation among pup mass at birth (day 0), but litters of five pups show, on average, a decline in within litter variation twice as great as do 4-

pup litters by age 15 days (change in RDPM, $\overline{X} \pm$ SD: 4-pup litters: -0.040 $\pm$ 0.085; 5-pup

litters: -0.091 $\pm$ 0.058).

Litter reductions occurred in half of the 12 restricted litters. Two 4-pup litters lost three

pups, while a third lost two. Three 5-pup litters had two pups eliminated. Most (four of six)

litter reductions took place prior to 12 d. For each of these reduced litters, growth of the

surviving littermates rebounded to growth rates similar to ad libitum litters while those pups

from intact litters lagged behind (Table III).

Discussion

For most small mammals, mothers are income breeders (Clutton-Brock 1991, Stearns 1992),

converting their current food intake into usable forms needed for gestation and lactation.

Such clearly seems to be the pattern for nursing grasshopper mice: mothers on restricted diets

could not compensate for reduced food availability by using stored energy reserves to cover

the needs of their suckling pups. Mothers on the restricted diet lost less than 4 g (< 10% of

mass at conception), on average, but the rationing strongly curbed pup growth, indicating that

my intended manipulation of food shortages to pups had succeeded. Further, the restriction

regime targeted pups during their period of dependence on milk. Growth rates following pups

switching to solid food (> 18 d) converged between treatment groups. This result is not solely

due to restricted litters catching up with ad libitum counterparts after being supplemented with

food following 18 days because ad libitum litters also showed significant growth increases (in

all three litter sizes) after the initiation of weaning. Mothers did not seem to adjust the

frequency of milk let-downs to either litter size or diet level, though there was a mild increase in MLD rate as pups grew older and larger. Thus, it appears logically that mothers in good condition must provide pups with somewhat greater quantities of milk during an average let-down rather than increasing the frequency. Further, consecutive MLDs are temporally unpredictable, presumably diminishing the possibility of pups being able to take strategic advantage of being able to anticipate MLD in an effort to gain more milk than its sibs (as can be done by some nestling birds starting to beg first).

As in other rodent studies (e.g. Fuchs 1982, Sikes 1995, Moodie 1999), mean pup growth was inversely related to litter size, and positively to maternal food access (Sikes 1995, 1996, Moses et al. 1998), indicating that competition can be shaped by either the demand (litter size) or supply (maternal food) functions. At face value, one would expect the combined effects of litter size and maternal food restriction to be most acute in the largest-litter: lowest-food (restricted 5-pup) treatment. As expected, pup growth was slowest for nurslings in these largest litters, but the frequency of litter reductions did not accord with this view. Reductions in 5-pup litters generally trimmed family size by 40%, to three pups, while mothers of 4-pup litters culled by 50-75%. In nine out of 11 litter reduction events, the timing of the infanticide happened prior to the typical growth rate decline that occurs ($\geq$ 12 d). These patterns suggest that individuals in 4-pup litters were somehow more susceptible to food shortages than those in 5-pup litters. Individuals from 4-pup litters are, on average, larger than pups from 5-pup litters and thus require more food (Moses et al. 1998). A diet restriction may more severely affect these larger pups, increasing the likelihood of death. Secondly, the intensity of competition for milk may be greatest in those litters where variation among siblings is least,

causing a greater number of individuals to perish during the food shortage. Experimental manipulation of pup-mass variance within litters will directly test this assertion.

On average, success rate of nipple-switching was greatest for individuals of 3-pup litters, followed by those of 4-pup litters, and lowest for 5-pup litters. This is unsurprising given that the number of unused (available) nipples is inversely related to litter size. More revealing is that only individuals in 4-pup litters showed a relationship between number of unused nipples gained per milk let-down and their daily growth. Nipple-switching patterns of pups from 3-pup litters may not relate to growth rates for two reasons: (1) such pups may never experience real shortages of milk, each have an average of two nipples per MLD; and (2) the variance in such litters will be naturally low (by nature of there being 'seconds' for everyone). The first part of this hypothesis is supported by the observed mean nipples gained by individuals in 3-pup litters, compared to the maximum average each pup could gain (1.54:2.00): pups in these smallest litters clearly do not even consume everything available to them. In four-pup litters, by contrast, the second part of the dynamic could become very important, polarizing the litter into relative 'haves' and 'have-nots' if some individuals are consistently better at getting a second nipple than others. The difference among rates of nipples-gained per pup reached its highest score within one four-pup litter (0.62). With six nipples, a four-pup litter not only has a lower maximum mean per pup (1.50) but also the highest potential variance (because the most usual pattern for a given MLD will be two pups having a score of 2 and the other two having a score of 1). If early 'winners' grow stronger and faster as the result of their early success, a positive feedback loop may propel them toward continuing relative success. Accordingly, one might expect variance to be much lower in 5-pup litters, where only one pup will get 'seconds' and, given the extra rival, it will have to be quite lucky to do so consistently

54

enough to enjoy a long-term growth effect, especially considering the overall energetic stress its mother is likely to be experiencing. Though sample sizes are small here (n's = 4), ad libitum litters demonstrate this pattern. Variance among pup masses declined over time at a rate twice as much in 5-pup litters as in 4-pup litters.

In conclusion, this study has demonstrated that nipple-switching operates in a non-domesticated mammal and may have considerable potential for shaping growth and survival for the young rivals that practice it. The resulting patterns of offspring welfare are likely to be affected within this context by both the maternal food budget and by the ratio of hungry mouths to milk outlets.

Acknowledgments

References

Bateman, N. 1957. Some physiological aspects of lactation in mice. Journal of Agriculture Science, 49, 60-77.

Cramer, C. P. & Blass, E. M. 1983. Mechanisms of control of milk intake in suckling rats. American Journal of Physiology, 245, R154-159.

Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton: Princeton University Press.

Day, C. S. D. & Galef, B. G. 1977. Pup cannibalism: one aspect of maternal behavior in golden hamsters. Journal of Comparative Physiological Psychology, 91, 1179-89.

Frank, L. G., Glickman, S. E. & Licht, P. 1991. Fatal sibling aggression, precocial development, and androgens in neonatal spotted hyaenas. Science, 252, 702-704.

Fraser, D. & Thompson, B. K. 1991. Armed sibling rivalry among suckling piglets. Behavoral Ecology and Sociobiology, 29, 9-15.

Fuchs, S. 1982. Optimality of parental investment: the influence of nursing on reproductive success of mother and female young house mice. Behavoral Ecology and Sociobiology, 10, 39-51.

Gilbert, A. N. 1984. Mammary number and litter size in Rodentia: the "one-half rule". Proceedings of the National Academy Science, 83, 4828-4830.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I Journal of Theoretical Biology, 7, 1-16.

Krackow, S. 1993. The effect of weaning weight on offspring fitness in wild house mice (Mus musculus domesticus): a preliminary study. Ethology, 95, 76-82.

McCarty, R. 1978. Onychomys leucogaster. Mammalian Species 87:1-6.

56

McClure, P. A. 1981. Sex-biased litter reduction in food-restricted wood rats (Neotoma floridana). Science, 211, 1058-1060.

Mock, D. W. & Parker, G. A. 1997. Evolution of Sibling Rivalry. Oxford: Oxford Press.

Mock, D. W. & Parker, G. A. 1998. Siblicide, family conflict and the evolutionary limits of selfishness. Animal Behaviour, 56, 1-10.

Moodie, J. D. 1999. Sibling rivalry for milk in northern grasshopper mice (Onychomys leucogaster). Ph.D. thesis. Norman: University of Oklahoma.

Moses, R. A., Boutin, S. & Teferi, T. 1998. Sex-biased mortality in woodrats occurs in the absence of parental intervention. Animal Behaviour, 55, 563-571.

Sibly, R. M. & Calow, P. 1986. Physiological ecology of animals. Oxford: Blackwell Scientific.

Siegel, S. & Castellan, N. J., Jr. 1988. Nonparametric Statistics for the Behavioral Sciences. 2nd edn. New York: McGraw-Hill.

Sikes, R. S. 1995a. Costs of lactation and optimal litter size in northern grasshopper mice (Onychomys leucogaster). Journal of Mammalogy, 76, 348-357.

Sikes, R. S. 1995b. Maternal response to resource limitations in eastern woodrats. Animal Behaviour, 49, 1551-1558.

Sikes, R. S. 1996a. Tactics of maternal investment of northern grasshopper mice in response to postnatal restrictions of food. Journal of Mammalogy. 77, 1092-1101.

Sikes, R. S. 1996b. Effects of maternal nutrition on post-weaning growth in two North American rodents. Behavioral Ecology and Sociobiology, 38, 303-310.

Sokal, R. R. & Rohlf, F. J. 1995. Biometry. 3rd edn. New York: W. H. Freeman.

Stearns, S. C. 1992. The evolution of life histories. New York: Oxford University Press.

Table I

Autocorrelation analysis of sequential MLD intervals for 24 grasshopper mouse mothers.

Data inclusive from nursing bouts of four different age periods (6-8 d, 9-11 d, 12-14 d, 15 d).

Litter ID	n	r	P
101	18	-0.34	0.16
103	11	-0.38	0.24
108	13	-0.44	0.13
109	13	0.23	0.44
110	13	0.13	0.61
111	17	-0.37	0.17
112	15	-0.18	0.31
119	25	0.13	0.56
121	24	0.15	0.57
162	17	0.20	0.43
163	18	-0.19	0.43
164	19	0.30	0.11
172	31	< 0.01	0.99
176	41	0.03	0.88
190	29	0.13	0.59
194	20	-0.23	0.46
195	34	0.07	0.71
301	31	0.26	0.15
302	8	0.06	0.89

(Table I continued)

303	14	0.34	0.24
305	31	0.10	0.61
306	24	0.02	0.91
307	11	-0.27	0.41
310	16	0.02	0.93

Table II

Relationship between mean number of nipples gained per pup per hour of nursing and mean pup growth rate for 24 litters for different ages. Reduced litters not included in analysis.

Age (d) observed suckling	Growth Period (d)	n	R^2	F	P
6-8	6-9	23*	< 0.01	0.10	0.75
9-11	9-12	20	< 0.01	0.01	0.94
12-14	12-15	18	0.35	8.56	0.01
15	15-18	18	0.02	0.30	0.59

* no milk let-downs were observed for one litter.

Table III

Daily growth rates ($\overline{X} \pm 95\%$ CI) at different ages for pups in larger litters (litter sizes of four and five combined). Litters are from Ad Libitum mothers, Restricted mothers where litter size reduced from 4 or 5 to ≤ 3, and Restricted mothers where litter size was not reduced. P values are calculated from t-tests (day 6) or one-way ANOVAs. Numbers in parentheses are sample sizes.

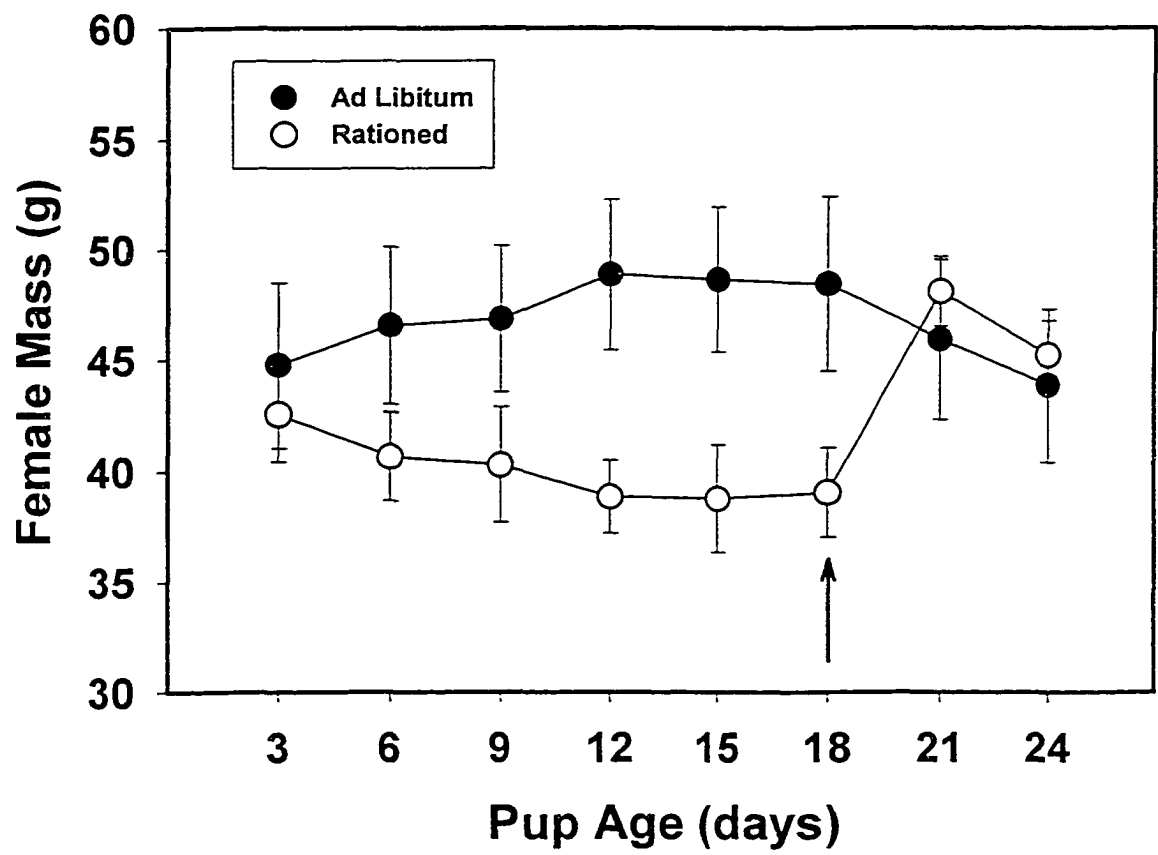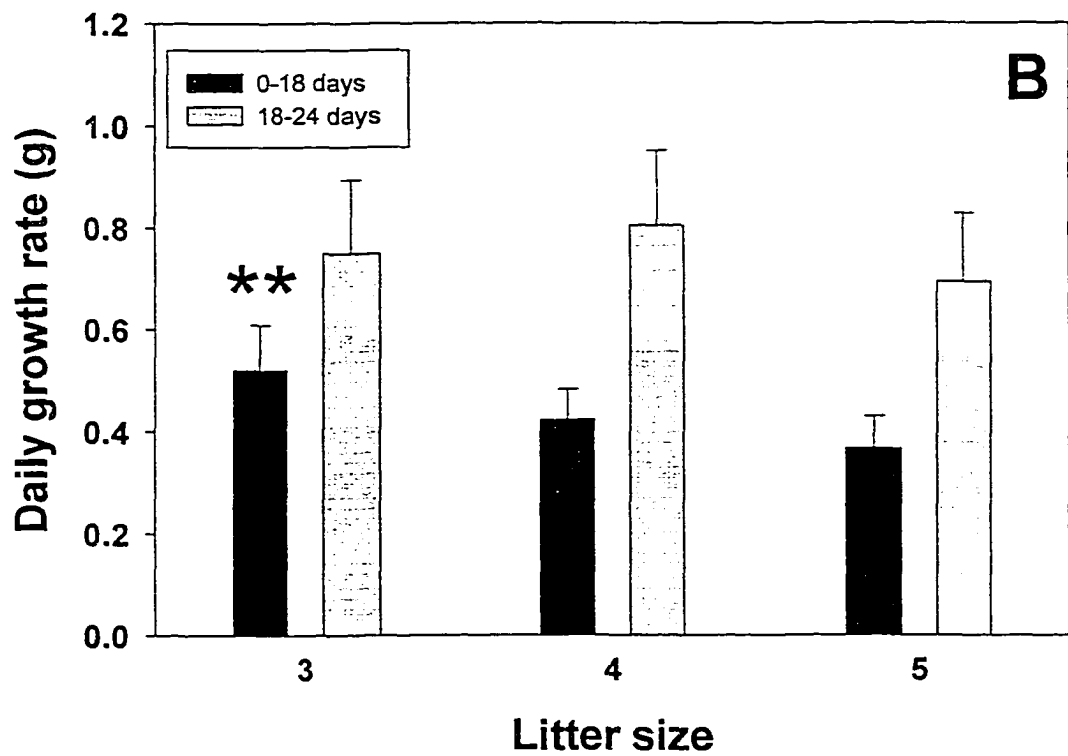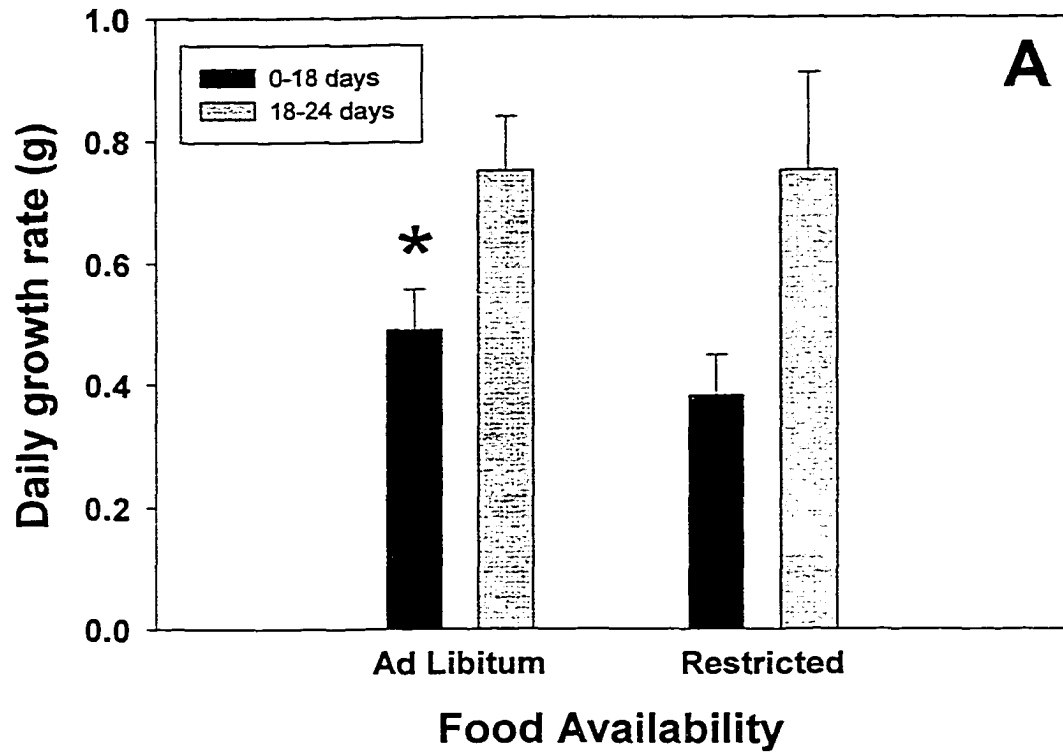| Age	Ad Libitum	Restricted-Reduced	Restricted-Intact	P
6	0.52 ± 0.09	—	0.36 ± 0.07	0.02
	(8)		(8)	
9	0.52 ± 0.05	0.33 ± 0.07	0.22 ± 0.08	< 0.001
	(8)	(4)	(4)	
12	0.48 ± 0.06	0.36 ± 0.21	0.33 ± 0.10	0.26
	(8)	(5)	(3)	
15	0.45 ± 0.05	0.47 ± 0.20	0.24 ± 0.08	0.26
	(8)	(6)	(2)	
18	0.30 ± 0.07	0.58 ± 0.19	0.18 ± 0.08	0.01
	(8)	(6)	(2)	

Figure Legends

Figure 1. Mean ($\pm$ 95% CIs) mass for lactating females on ad libitum or restricted (75% of ad libitum) diets over the age of their pups. Arrow represents the day restricted females were returned to ad libitum food. Repeated measures MANOVA for ages 0 to 18: food availability, $F_{1, 18} = 8.99$, P = 0.008; time-food availability interaction, $F_{5, 14} = 8.19$, P < 0.001.
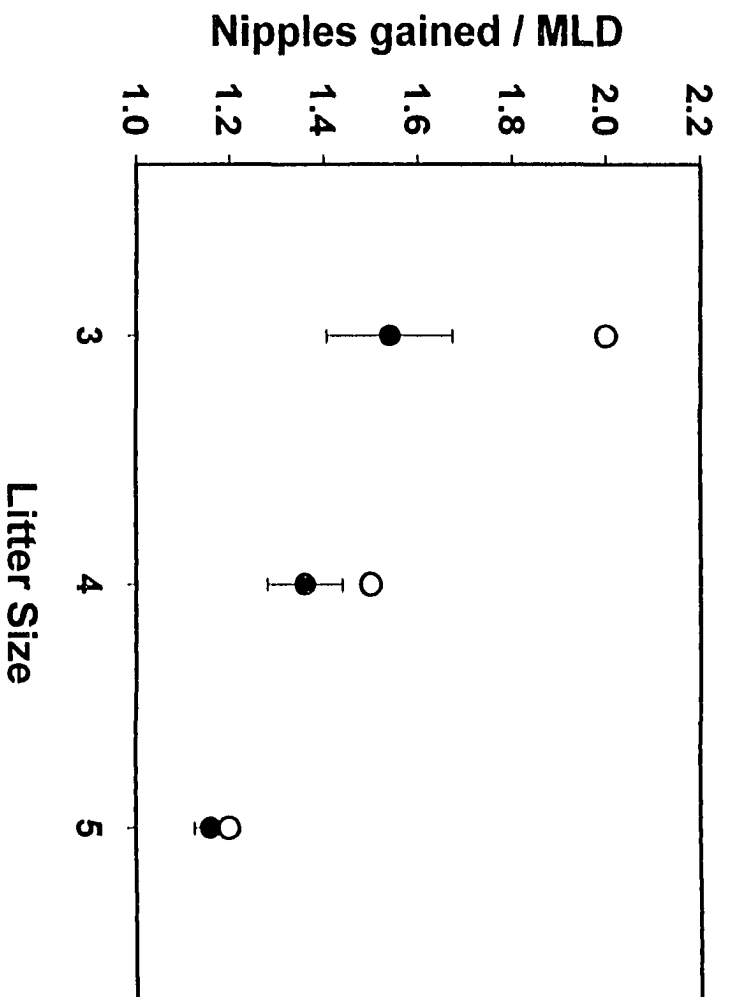
Figure 2. Mean ($\pm$ 95% CIs) daily growth rate of pups for first 18 days of age and 18-24 days of age. A. Pup growth when mother given ad libitum vs. restricted diet (ANOVA days 0-18: $F_{1, 18} = 11.02$, $\underline{P} = 0.004$; ANOVA days 18-24: $F_{1, 18} = 0.06$, $\underline{P} = 0.80$). Asterisk represents significant difference between 0-18 day treatments. B. Pup growth for litter sizes 3, 4 and 5 (ANOVA days 0-18: $F_{2, 18} = 7.19$, $\underline{P} = 0.005$; ANOVA days 18-24: $F_{2, 18} = 0.51$, $\underline{P} = 0.60$). Double asterisk represents difference between litter sizes 3 and 5 using Tukey-Kramer multiple comparison tests.
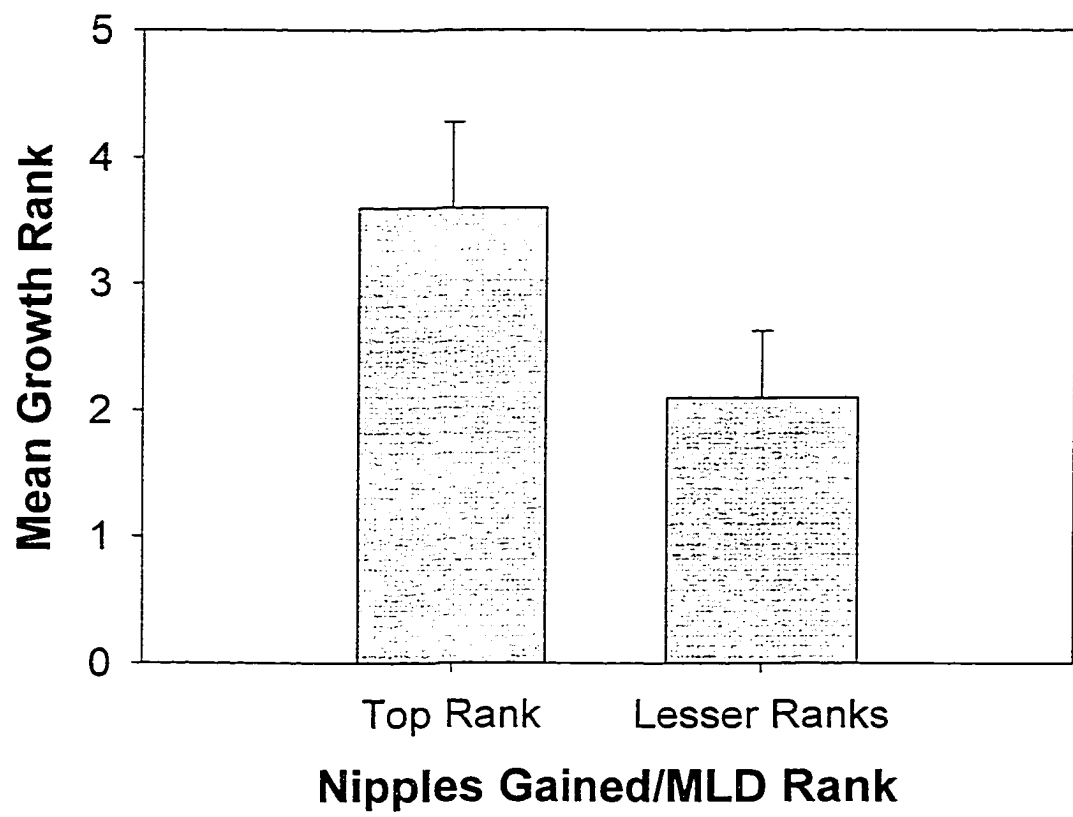
Figure 3. Mean ($\pm$ 95% CIs) number of nipples gained per pup per milk let-down (MLD) for three litter sizes (closed circles). Open circles represent mean nipples per pup within a litter if all nipples are occupied for each milk let-down. Only intact litters included in analysis. ANOVA: $F_{2, 12} = 10.29$, P = 0.003.

Figure 4. Relationship between rank of nipples gained per MLD and daily growth-rate rank for first 18 days of age for individual pups from 4-pup litters. Higher number refers to higher rank: Wilcoxon signed rank test: T = 7.5, N = 5, P = 0.03.

GENDER-BASED SUCKLING BEHAVIOUR AND GROWTH PATTERNS IN NORTHERN GRASSHOPPER MICE (ONYCHOMYS LEUCOGASTER)

Running headline: Moodie: grasshopper mouse suckling behaviour

JAMES D. MOODIE

University of Oklahoma

Norman

Correspondence:

Department of Biological Sciences

Idaho State University

Pocatello, ID 83209

moodjame@isu.edu

word count: 2,980

Abstract

Biased investment in offspring of one sex is predicted whenever parents expect a greater fitness return from the favoured sex. Such bias may occur due to gender differences in variance of future reproductive success, early body size dimorphism, or dispersal patterns. I investigated whether northern grasshopper mouse (<u>Onychomys</u> <u>leucogaster</u>) parental investment is skewed to one sex prior to the onset of weaning. I observed the suckling behaviour of twenty-four litters ranging in size from three to five pups. Suckling measures included sucking duration following a milk let-down, attempted number of nipple-switches, search duration, and nipple-switching success. I found no indication of gender bias for birth sex ratio, pup mass, or behavioural measures of suckling, except for a trend where male pups use the hind nipple pair more often than their sisters do. There also was no tendency for mothers to adjust sex ratios in favour of one sex via infanticide when faced with limited food resources. In this species, mothers and pups may have little incentive to direct maternal investment towards one sex at the expense of the other.

Introduction

Parental decisions over resource allocation are predicted to be influenced by offspring gender (Willson & Pianka 1963). Generally, any bias toward one sex is expected when investment in the favoured sex provides greater returns to the parental fitness (Fisher 1958; Trivers & Willard 1973; Maynard Smith 1980). Parental decisions include skewing the sex ratio toward one sex, depending on current or projected parental conditions (Trivers & Willard 1973), or preferentially investing resources in that sex after conception (Stamps 1990). On the other hand, physiological and/or genetic constraints may limit the options available to parents.

For mammals, chromosomal mechanisms of sex determination constrain sex allocation strategies primarily to post-zygotic manipulation (Godfray & Werren 1996). Mothers have opportunities during pre- and post-natal care to invest or even eliminate individuals differentially according to sex, and are predicted, when in good condition, to favour the sex in which variance in reproductive success is greater thereby producing more competitive individuals of this sex (Trivers & Willard 1973). For mammals, where polygyny is common, males tend to have greater reproductive variance (see Clutton-Brock 1988). Clutton-Brock & Albon (1982) identified two other factors influencing mammalian parental investment allocation beyond the parent's personal condition. First, the fitness payoff associated with offspring sex should depend on how parental care affects the reproductive success of each sex. If one sex benefits more than the other does from the same amount of investment, it is advantageous for parents to bestow additional care to that sex. Second, post-weaning investment must be included in total investment provided to young. Thus, it is possible for one sex to be favoured prior to weaning, but then to have a post-weaning reversal in parental care that either eliminates any net effect or, in some cases, tips true parental investment bias

70

the other way. If, for example, male red deer (Cervus elahpus) disperse shortly after weaning, but females remain on the mother's home range, pre-weaning investment may be skewed toward male offspring while females reap the benefit of post-weaning maternal resources (Clutton-Brock et al. 1982).

In polygynous species, male mating success (hence, expected fitness) varies more so than female reproductive success, resulting in males competing for mating opportunities while females provide most if not all the maternal care (Trivers 1972). Alternatively, differential maternal investment is predicted if sexes differ in dispersal patterns, such that the sex dispersing farther from the natal home range may require additional resources prior to dispersal to enhance its success, while a mother may reduce her future reproductive success subsequent to weaning by sharing her range with the other sex (via 'local resource competition': Clark 1978). Sexual dimorphism can also influence differences between the costs of raising male and female offspring, which may affect resource allocation (Willson & Pianka 1963). Sex-biased allocation has been documented in some polygynous and/or sexually dimorphic mammals (reviewed in Clutton-Brock et al. 1981; Clutton-Brock 1991). By contrast, few data exist for monomorphic and/or the relatively few monogamous mammalian species (Kleiman 1977; Clutton-Brock & Iason 1986; Clutton-Brock 1989; Clutton-Brock 1991). For these mammals, sex differentiated growth patterns among offspring may be the exception rather than the rule given that there is little sexual dimorphism. However, because these species are not expected to deviate from unity in mortality or resource allocation, there may be a bias for not reporting or publishing nonsignificant results (Festa-Bianchet 1996).

Northern grasshopper mice (<u>Onychomys</u> <u>leucogaster</u>) differ from those mammalian species known to demonstrate sex-biased parental investment in a number of ways. Based on field trapping, semi-natural and laboratory observations, the species appears to exhibit a seasonally monogamous mating system with male and female mates forming extended pair-bonds (Egoscue 1960; Ruffer 1965). However, laboratory observations of male copulatory behaviour suggest otherwise (Lanier & Dewsbury, 1977; Dewsbury, 1981), and evidence showing that oestrous females prefer the odours of mature males raised by mothers with access to abundant food over males raised by mothers with somewhat limited resources (Moodie 1999), suggest that males may have greater variance in mating success. As well, grasshopper mouse pup growth rates appear not to vary between sexes from birth through weaning (Sikes 1996b). And after weaning, gender mass differences have been reported by 10 weeks of age for some captive groups (Pinter 1970), but no sexual dimorphism has been detected in adult individuals in other laboratory populations (Dewsbury et al. 1980; Sikes 1996a; Moodie 1999). Gender biased dispersal patterns are unknown for these mice. Finally, though sexes may have similar masses throughout dependency on milk, each sex may differ in how milk is obtained. For example, one sex may be able to drain a nipple of milk more efficiently, while the other sex may be better at exploiting more than one nipple (i.e. nipple-switching: Moodie, 1999) per milk let-down event. Similarly, how mammalian young allocate ingested milk for growth, development and maintenance (e.g. muscle, fat, other tissues) may be sex dependent (Byers & Moodie 1990; Clutton-Brock 1991).

Here I report on a laboratory study investigating differential maternal investment and sex differences in the suckling behaviour of pre-weaning northern grasshopper mice. The experimental protocol was designed specifically to test for: 1) skewed sex ratios based on

maternal body condition and litter size, 2) maternal investment bias towards one sex when faced with limited resources, and 3) gender differences in measures of suckling behaviour. Grasshopper mice, because of their monomorphic size and similar gender-based growth rates, are predicted to show no sexual-bias in litter size adjustment or pup behavior.

Methods

I established a breeding colony of northern grasshopper mice at the University of Oklahoma's Zoology Department live animal facility in Norman. Grasshopper mouse females possess six mammae, and, in this study, gave birth to litters ranging in size from three to five (litter sizes hereafter abbreviated as 'LS-3', 'LS-4' and 'LS-5'). Gender data were collected on 24 litters while studying other aspects of sibling behaviour (see Moodie 1999 for details). If litter size of a litter changed during the study (see below), I treated the altered litter as an independent sample. Mothers were randomly assigned to either ad libitum or food-restricted diets (ca 75% of the amount consumed by lactating females fed ad lib: see Moodie 1999 for further details).

Neonates, when discovered (< 24 h old), were weighed individually to the nearest 0.1 g on an electronic balance, then sexed (using ano-genital distances) and uniquely marked using a 26 gauge hypodermic needle to inject a small tattoo of India ink under the skin on one or two legs near the junction with the body. Because tattoos ceased to be visible after approximately seven days, I uniquely ear-clipped each pup on day three post-partum, removing approximately 1 mm^2 of pinna. To facilitate individual recognition during suckling bouts, I marked very young pups (i.e., lacking adequate hair) with unique white paint patterns on the head and belly. When pups had sufficient fur (approximately 9 days of age), I applied hair-dye (Lady Clairol

73

Platinum Blonde hair dye) to the pups on their dorsal sides creating unique patterns on each pup.

Grasshopper mouse mothers are known to practice infanticide in the laboratory (Sikes 1995a, 1996b, pers. obs.). To reduce this risk from handling, I did not begin weighing mothers until day three post-partum, and subsequently measured pups and mothers every three days thereafter. Individuals were not handled on other days unless necessary (see below).

On day six I began collecting behavioural data. To observe nursing in detail, I first transferred the mother to a clean, clear plastic cage devoid of bedding materials, but supplied with a opaque, cylindrical plastic nest-box (see Moodie 1999 for details). After an hour-long separation of pups from the mother, I reunited the litter and mother in this new 'observation' nest-box. To view the mother's ventral surface and pups closely, I elevated the cage and nest-box above an angled mirror and observed from behind a screen. When the mother stopped moving sufficiently to allow suckling, I began recording behavioural events, either directly or using a videotape camera for 1-2 h. This procedure was repeated once every three days through day 15 (four samples per litter). Milk let-downs (hereafter MLD) are easily detectable by observing the female arch her back followed by the stereotypical pup sucking response (Drewett et al. 1974). If no MLDs were recorded during a given sampling period, I returned the mother and her litter to the observation cage the following day and observed or taped a substitute session. I ended weaning on day 25, placing each litter in a cage separate from the mother.

Grasshopper mice have three nipple pairs: one pectoral, and two abdominal (labeled as front, middle, and hind nipples: McGuire 1998). Just prior to a milk let-down, I recorded the nipple (row and left vs. right) to which each pup attached. Suckling duration was measured beginning at the time of milk let-down, identified by the stereotypic 'reflex' behavior of the mother (Drewett et al. 1974) followed by the initiation of the 'stretch' response by the pup (Lincoln et al. 1973), until the pup released the nipple it currently occupied. I measured search duration as the number of seconds a pup remained unattached to any nipple following release of the first nipple. A successful nipple-switch was defined as a pup moving from its original nipple to one that was previously unoccupied.

Although I used a much less restrictive restricted diet than those featured in other rodent food-limitation experiments (ca 80% of a nonlactating diet: McClure 1981; Sikes 1995b; 1996a, b; Moses et al., 1998), some of the food restricted litters suffered partial litter loss (six of 12 food-restricted litters vs. zero of 12 ad lib. litters). To limit the number of pup deaths during the experiment, I used only four litters per treatment cell (litter size x food availability). Though it was not possible to watch all pups continuously around the clock, I did happen to observe five cases in which mothers that were fed ad libitum (females not included in this study) fatally bit their own pups. Sikes (1996a, b) also reported female grasshopper mice actively committing infanticide. In the five cases I observed, victims were killed almost instantaneously from a single bite.

I used parametric statistical tests for all analyses where appropriate. To conduct all tests, I used JMP software (1996 SAS institute, Version 3.2.1).

Results

Maternal mass at day three post-partum did not predict litter size (logistic regression: N = 24, X^2 = 0.21, df = 1, P = 0.64). Sex ratios at birth for the three litter sizes did not vary from parity, nor from each other ($\overline{X}$ proportion of males ± 95% CI: LS-3: 0.50 ± 0.18, LS-4: 0.44 ± 0.22, LS-5: 0.45 ± 0.12; $F_{2,21}$ = 0.13, P = 0.87). Though the study was not intended to induce litter reduction, six litters suffered partial mortality (three each of food-limited LS-4 and LS-5). For these six litters, females out-numbered males 15 to 12 and nine of the 14 fatalities were female. However, in the nine cases where the pre-reduction event sex ratio was skewed toward one sex, pup mortality was not biased against females, but against the more prevalent sex (Fisher's Exact test: N = 9, P = 0.05). When the sex ratio prior to a pup fatality was biased towards males (N = 4), a male died in three of four cases, while when females outnumbered males (N = 5), a female perished each time. Mass of pups did not differ between the sexes for any age from birth through day 24 either when considering only the 18 intact litters (MANOVA: Wilk's lambda = 0.007, $F_{1,14}$ = 1.94, P = 0.19) or when only food-restricted litters that remained intact were analyzed (MANOVA: Wilk's lambda = 0.89, $F_{1,5}$ = 0.60, P = 0.47).

I assessed several behavioural parameters across litters, including nipple attachment, nipple-switching, switching success, sucking duration and nipple search rates for each sex. Nipple attachment rates (mean number of MLDs in which an individual was attached to a nipple at the time of MLD for each sex) across all MLDs, regardless of age, were nearly identical ($\overline{X}$ ± 95% confidence interval: females: 0.937 ± 0.035; males: 0.931 ± 0.044; paired t_{27} = 0.15, $\underline{P}$ = 0.88). Similarly, pups of each sex attempted nipple switches in equal proportions (females: 0.732 ± 0.053; males: 0.752 ± 0.059; paired t_{26} = -0.48, P = 0.63) and mean suckle duration

time prior to switching was equivalent (females: 29.11 ± 11.39 s; males: 27.73 ± 10.73 s; paired t_{26} = 1.02, P = 0.32). Though female and male pups attempt nipple switches at equal rates, it is possible that one sex will have been more likely to succeed in finding an unused nipple. Again, this was not found (females: 0.566 ± 0.089; males: 0.583 ± 0.096; paired t_{26} = -0.34, P = 0.73). Additionally, mean search duration times for second nipples did not differ between females and males (females: 52.99 ± 9.97 s; males: 55.40 ± 10.04 s; paired t_{26} = -0.34, P = 0.73). Therefore, none of the behavioural parameters associated with obtaining milk differed according to sex.

One behavioural pattern tended to separate males from females: nipple-pair usage. Male offspring were more likely to be attached to one of the hind pair of nipples at the time of milk let-down than were female offspring among 85 pups that survived to weaning (females: n = 45, 30.6 ± 3.6%; males: n = 40, 35.8 ± 3.5%; t_{83} = 2.04, P = 0.05). No difference in attachment to other nipple-pairs was detected (middle nipple-pair: t_{83} = 0.53, P = 0.60; front nipple-pair: t_{83} = 1.46, P = 0.15). However, when I controlled for within-litter variation by computing a mean percent usage for each sex within a litter, the difference in percent usage for the hind nipple-pair was not significant for 20 litters containing both male and female pups (male-female difference: 4.2 ± 2.3%, paired t_{19} = 1.87, P = 0.08).

Discussion

Northern grasshopper mice show little evidence that maternal investment is biased towards one sex over the other. There was no trend for a skew in birth sex ratios according to litter size, and lactating mothers faced with limited food did not favour either sex when trimming litter size. Growth for each sex was similar throughout the lactation period, a pattern that

apparently continues into adulthood (Sikes 1996a). Sexual differences in behaviors used to obtain milk were lacking for a number of measures. Though there was a slight tendency for males to occupy hind nipples more often than females, all nipple pairs were used by pups within a litter across litters. This was true even when litter size was small ($\leq$ 3), suggesting that milk quantities are probably similar across nipples though this has not been assessed directly. There is no indication that males are more active in nipple-switching scrambles for extra milk. First-nipple suckling duration was equivalent, searching duration did not differ, and males were no more likely to attempt a nipple switch or to gain an unused nipple when switching than females.

The lack of sexual bias in both maternal investment and sex differences in suckling behaviour reported here concurs with the findings of Sikes (1996b), who found no difference in consumption of resources by sex among grasshopper mouse litters, even when food was more severely limited to mothers. However, while Sikes observed that nearly all food-stressed mothers eliminated their entire litters, I observed partial-litter or no reductions in response to a milder food restriction regime (Sikes 1996b: ca. 80% of a non-lactating vs. present study: ca. 75% of a lactating female's diet). The present study lends support that maternal infanticide is a function of the mother's body condition, litter size and available food resources. Maternal infanticide is known to occur in other rodents, where it may also serve an adaptive litter-trimming role (e.g., Day & Galef 1977). Therefore, the present study would seem to provide a better opportunity to detect preferential treatment towards one sex, had such existed. These results urge further study of sex ratio manipulations by rodent females under mild food-restriction conditions compared with previous studies (McClure 1981; Sikes 1995b; 1996a, b; Moses et al. 1998).

The lack of differences between male and female pups' suckling behaviours suggests that mixed-sex litters probably do not differ in energy demands from comparable litters composed of only one sex as has been shown for size-monomorphic birds (Voltura 1998). If true, mothers have little incentive to bias investment towards one sex based on offspring investment costs and current food-resource conditions. Additionally, mothers may not be able to discriminate against one sex because of strategies used to disperse milk to young. While some mammals have specific teat orders (reviewed in (McGuire 1998), grasshopper mouse pups frequently switch nipples following discrete milk let-downs where milk appears to be available to all nipples, and most likely does not measurably differ in quantity. Teat orders exhibited by prairie voles, Microtus ochrogaster (McGuire 1998), suggest that mothers are manipulating milk supply to specific nipples, a source of variation that could foster sibling competition. Though sex differences are not reported for voles, it would be interesting to determine if one sex is predominately attached to the rear nipples given that this species exhibits sexual size dimorphism (Dewsbury et al. 1980). For many species of birds (reviewed in Mock & Parker 1997), discrepancies in gender growth rates further complicate the dynamics of sibling-sibling and parent-offspring interactions. In the grasshopper mouse nursing system, it is possible that similar milk quantities among mammary glands and nipple-switching behaviour will result from selection for monomorphic growth patterns in this species. A comparison of suckling patterns with other rodents exhibiting variations in size dimorphism is desirable to determine if teat order and nipple-switching suckling strategies are general solutions to competitive situations.

79

There is no evidence that when mothers commit infanticide in this species, there is preferential bias against either sex. Though sons raised by food stressed mothers may be in the future less desirable as mates, and therefore less likely to breed (Moodie 1999), mothers did not selectively kill sons. It is possible that reproductive success of daughters is just as likely to be negatively affected by rearing conditions. Food-stressed juvenile female hamsters (Mesocricetus auratus), for example, produce female-biased litters, and any sons produced are small when compared to the male offspring of well-nourished mothers (Huck et al. 1986; Huck et al. 1987). Smaller males may be unable to contend for mates in the presence of larger competitors (Krackow 1993). Similar to this study, Sikes (1995b, 1996a) found sex-biased mortality in neither eastern woodrats (Neotoma floridana) nor northern grasshopper mice. However, Moses et al. (1998) did find male-biased mortality for the sexually dimorphic (males > female) bushy-tailed woodrats (N. cinerea), but found no evidence that mothers selectively discriminated against sons. In this case, male-biased mortality of bushy-tailed woodrats appears due to greater caloric demand by growing males.

The present study suggests that mothers of other monomorphic mammalian species may not differentiate between sexes during pre-weaning investment, and, in fact, this investment parity should be expected (Trivers & Willard 1973; Festa-Bianchet 1996). However, it is unclear if equivalent growth and behaviour is due to maternal control of resource allocation or to similar competitive abilities of male and female offspring. Experimental approaches manipulating sex ratios and size variation within litters will shed light on parent-offspring interactions during lactation.

Acknowledgements

References

Clark, A. B. 1978. Sex ratio and local resource competition in a prosimian primate. Science, 201, 163-165.

Clutton-Brock, T. H. (ed). 1988. Reproductive success. Chicago, IL: University of Chicago Press.

Clutton-Brock, T. H. 1989. Mammalian mating systems. Proceedings of the Royal Society London, Series B, 236, 339-372.

Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton, NJ: Princeton University Press.

Clutton-Brock, T. H. & Albon, S. D. 1982. Parental investment in male and female offspring in mammals. In: Current Problems in Sociobiology (Ed. by Group, K. s. C. S.), pp. 223-247. Cambridge: Cambridge University Press.

Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. 1981. Parental investment in male and

female offspring in polygynous mammals. Nature, 289, 487-489.

Clutton-Brock, T. H., Albon, S. D. & Guinness, F. E. 1982. Competition between female

relatives in a matrilocal mammal. Nature, 300, 178-180.

Clutton-Brock, T. H. & Iason, G. R. 1986. Sex ratio variation in mammals. Quarterly Review

of Biology, 61, 339-374.

Day, C. S. D. & Galef, B. G. 1977. Pup cannibalism: one aspect of maternal behavior in golden

hamsters. Journal of Comparative Physiological Psychology, 91, 1179-1189.

Dewsbury, D. A. 1981. The coolidge effect in northern grasshopper mice (Onychomys

leucogaster). Southwest Naturalist, 26, 193-197.

Dewsbury, D. A., Baumgardner, D. J., Evans, R. L. & Webster, D. G. 1980. Sexual

dimorphism for body mass in 13 taxa of muriod rodents under laboratory conditions.

Journal of Mammalogy, 61, 146-149.

Drewett, R. F., Statham, C. & Wakerley, J. B. 1974. A quantitative analysis of the feeding

behavior of suckling rats. Animal Behaviour, 22, 907-913.

Egoscue, H. J. 1960. Laboratory and field studies of the northern grasshopper mouse. Journal

of Mammalogy, 41, 99-110.

Festa-Bianchet, M. 1996. Offspring sex ratio studies of mammals: does publication depend

upon the quality of the research or the direction of the results? Ecoscience, 3, 42-44.

Fisher, R. A. 1958. The genetical theory of natural selection. Clarendon: Oxford.

Godfray, H. C. J. & Werren, J. H. 1996. Recent developments in sex ratio studies. Trends in

Ecology and Evolution, 11, 59-63.

Huck, U. W., Labov, J. B. & Lisk, R. D. 1986. Food-restricting young hamsters (Mesocricetus auratus) affects sex ratio and growth of subsequent offspring. Biology of Reproduction, 35, 592-598.

Huck, U. W., Labov, J. B. & Lisk, R. D. 1987. Food-restricting first generation female hamsters (Mesocricetus auratus) affects sex ratio and growth of third generation offspring. Biology of Reproduction, 37, 612-617.

Kleiman, D. G. 1977. Monogamy in mammals. Quarterly Review of Biology, 52, 39-69.

Krackow, S. 1993. The effect of weaning weight on offspring fitness in wild house mice (Mus musculus domesticus): a preliminary study. Ethology, 95, 76-82.

Lanier, D. L. & Dewsbury, D. A. 1977. Studies of copulatory behaviour in northern grasshopper mice (Onychomys leucogaster). Animal Behaviour, 25, 185-192.

Lincoln, D. W., Hill, A. & Wakerley, J. B. 1973. The milk-ejection reflex of the rat: an intermittent function not abolished by surgical levels of anaesthesia. Journal of Endocrinology, 57, 459-476.

Maynard Smith, J. 1980. A new theory of sexual investment. Behavioral Ecology and Sociobiology, 7, 247-251.

McClure, P. A. 1981. Sex-biased litter reduction in food-restricted wood rats (Neotoma floridana). Science, 211, 1058-1060.

McGuire, B. 1998. Suckling behavior of prairie voles (Microtus ochrogaster). Journal of Mammalogy, 79, 1184-1190.

Mock, D. W. & Parker, G. A. 1997. The Evolution of Sibling Rivalry. Oxford: Oxford University Press.

Moodie, J. D. 1999. Sibling rivalry for milk in northern grasshopper mice (Onychomys leucogaster). Ph.D. thesis, University of Oklahoma.

Moses, R. A., Boutin, S. & Teferi, T. 1998. Sex-biased mortality in woodrats occurs in the absence of parental intervention. Animal Behaviour, 55, 563-571.

Pinter, A. J. 1970. Reproduction and growth for two species of grasshopper mice (Onychomys) in the laboratory. Journal of Mammalogy, 51, 236-243.

Ruffer, D. G. 1965. Sexual behaviour of the northern grasshopper mouse (Onychomys leucogaster). Animal Behaviour, 13, 447-452.

Sikes, R. S. 1995a. Costs of lactation and optimal litter size in northern grasshopper mice (Onychomys leucogaster). Journal of Mammalogy, 76, 348-357.

Sikes, R. S. 1995b. Maternal response to resource limitations in eastern woodrats. Animal Behaviour, 49, 1551-1558.

Sikes, R. S. 1996a. Effects of maternal nutrition on post-weaning growth in two North American rodents. Behavioral Ecology and Sociobiology, 38, 303-310.

Sikes, R. S. 1996b. Tactics of maternal investment of grasshopper mice in response to postnatal restriction of food. Journal of Mammalogy, 77, 1092-1101.

Stamps, J. A. 1990. When should avian parents differentially provision sons and daughters? American Naturalist, 135, 671-685.

Trivers, R. L. 1972. Parental investment and sexual selection. In: Sexual Selection and the Descent of Man, 1871-1971 (Ed. by Campbell, B.), pp. 136-179. Chicago: Aldine.

Trivers, R. L. & Willard, D. E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science, 179, 90-92.

Willson, M. F. & Pianka, E. R. 1963. Sexual selection, sex ratio and mating system. American Naturalist, 97, 405-407.

MOTHER'S CONDITION DURING LACTATION INFLUENCES SON'S SEXUAL ATTRACTIVENESS IN GRASSHOPPER MICE

Moodie: grasshopper mouse mate choice

JAMES D. MOODIE

Department of Zoology

University of Oklahoma

Norman

Correspondence:

Department of Biological Sciences

Idaho State University

Pocatello, ID 83209

e-mail: moodjame@isu.edu

Word count: 3500

Abstract

Female mate choice, which is widely believed to be an important determinant of male reproductive success in some rodents, may be related to male body condition very early in life. I used northern grasshopper mice (Onychomys leucogaster) to test whether sons of mothers that had been experimentally food-stressed during lactation were less attractive after reaching sexual maturity than sons whose mothers had had free access to food while nursing their young. I conducted 16 choice tests for male odour using an Y-maze apparatus. Oestrous females visited sons of unstressed mothers more often and spent greater amounts of time in chambers associated with such males. Female sexual discrimination against sons of food-stressed mothers suggests that male reproductive success may hinge, at least in part, on adequate maternal investment. These results also suggest the potential for long-term fitness consequences of other traits that affect whether a neonate obtains sufficient early nutrition, including sibling competition for milk.

Introduction

Because polygyny is the rule rather than the exception in mammalian mating systems, males of most species are expected to vary more in reproductive success than are females (e.g., Clutton-Brock 1988). If early parental investment affects the future mating success of offspring, then mothers in good condition should invest preferentially in sons (Trivers and Willard 1973). The idea is that, once they have reached adulthood, sons raised by mothers in poor condition may be at a relatively greater disadvantage when competing for mates than daughters raised by handicapped mothers.

Evidence for this relationship exists for many mammals. Red deer (Cervus elaphus) calves born to mothers in poor condition during the previous rut are more likely to die (Clutton-Brock et al. 1982). A son's lifetime reproductive success rises sharply with maternal rank (which in turn is correlated positively with maternal condition), while no such relationship exists for daughters (Clutton-Brock et al. 1984). In primates, both survivorship (Meikle and Vessey 1988) and reproductive success (Paul et al. 1992) of sons vary positively with maternal condition. Various rodents show similar trends. The sons of hamster mothers that are experimentally food-deprived during gestation grow more slowly and attain lower adult mass than sons whose mothers are fed an ad. lib. diet while pregnant (Labov et al. 1986). If light males are disadvantaged in competition for territories and/or high-ranking status, as has been shown for wild house mice (Mus musculus, Krackow 1993), the potential for diminished male reproductive success is apparent. Inadequate nourishment prior to birth may be sufficient to reduce a male's reproductive success. Meikle et al. (1995) report that male house mice born to mothers that had been undernourished while pregnant are, as adults, less attractive to females.

However, because the lactation period carries an even greater energetic expense than gestation (reviewed in Thompson 1992, Sikes 1995a), and because it occurs just after the costs of pregnancy have been paid, mothers may be even more likely to fall short of offspring demand while nursing. By experimentally enlarging litter sizes or restricting maternal access to food during lactation, various authors have reported that offspring respond by growing more slowly and/or by reaching lighter weaning weights (Galler and Turkewitz 1975; König et al. 1988; Wright et al. 1988; Mendl and Paul 1989; Sikes 1995a, b; Moodie 1999). And while it has been shown that male weaning weight may impair performance in intra-sexual competition (Krackow 1993), the possible impact of early milk-deprivation on subsequent inter-sexual processes, especially active mate-choice by females, is unknown.

In some rodents, female choice may play a large role in determining a male's reproductive success (reviewed by Meikle et al. 1995) and male odour is commonly regarded as the chief source of proximate cues (Blaustein 1981, Huck and Banks 1982, Lenington 1983, Hurst 1990b, Drickamer 1992). Oestrous females seem capable of detecting male quality through olfaction (Thomas and Dominic 1988). If male quality is linked to the effects of early environmental conditions, females have a means of selecting high quality mates. I tested whether sons born to grasshopper mouse (Onychomys leucogaster) females that were food-stressed during the first 18 days post-partum (the primary period of lactation) were less sexually attractive to oestrous females than were sons born to mothers with free access to food throughout lactation. Attraction was determined through female behavioural responses to male odour.

Methods

Female choice-test subjects were either wild-caught (n = 5) or laboratory raised (n = 11). All had already given birth to at least one litter prior to participating in a mate choice trial, and in each case had been fertilized by an experienced male. Upon determining that a female was pregnant (based on abdominal palpation), I relocated her from the male's cage to a clean cage and checked daily for pups. Grasshopper mouse females exhibit a post-partum oestrus (Horner and Taylor 1968), so each was tested for male odour preference in an Y-maze testing apparatus shortly after her neonates were found. For three randomly chosen mothers, I confirmed oestrous state from the cornification of epithelial cells and absence of leucocytes of vaginal smears (Allen 1922). I tested each female only once.

Treatment males came from one of two groups. The first consisted of seven males raised by mothers that had had unlimited access to food (rat blocks) throughout pregnancy and lactation (hereafter 'Ad Lib' males). The other group of nine males had been raised by mothers that had received ca 75% of the amounts consumed by the unrestricted females from the moment of parturition (day 0) to 18 days post-partum. This is around the time pups begin ingesting solid food (hereafter 'Low Food' males). Mothers under this restricted diet lost approximately three grams (ca 7%) in body mass. A sample of wild caught reproductively active females averaged 38.9 g ± 4.34 SD (n = 14). The mean difference between the greatest and least body mass measurements of each of these females was nearly five grams, indicating that changes of three grams were well within natural mass fluctuations. Males ranged in age from 111 to 276 days old (sexual maturity is 56 days as reported by Pinter 1970), and were from natal litter sizes three, four, or five.

The Y-maze apparatus followed the design of Meikle et al. (1995). For each trial, one Ad Lib and one Low Food male were randomly selected from each treatment pool, then placed alone in a plastic container. Males were unrelated ($r < 0.125$). A test female, unrelated to either male, was placed into the central cage (foot of the Y), which had two choice cylinders (arms of the Y) inserted on opposite cage walls. A 60 cm length of rubber tubing connected each cylinder to just one of the male containers (random order), with a pump forcing air from the male containers into the choice cylinders. I used opaque partitions to screen all three individuals from having any visual contact with the others. After the female had had one minute to habituate to the apparatus, I recorded her location continuously for 15 minutes. For a female's position to be considered inside a choice cylinder, all four of her feet had to have left the central chamber floor. I then tabulated the number of visits and visit length to each cylinder. Because there were few males in each treatment group, I randomly paired males without regard to mass ($\overline{X} \pm SD$: Ad Lib: 41.0 ± 4.0 g; Low Food: 39.0 ± 5.3 g), age (Ad Lib: 166 ± 51.1 d; Low Food: 210 ± 41.3 d), or natal litter size (Ad Lib: 3.71 ± 0.95 Low Food: 4.11 ± 0.78). Except for one pair selected twice, I used a different combination of male-pairs for each test. Individual males were used one to four times (Ad Lib: 2.23 ± 0.49; Low Food: 1.78 ± 0.97).

When appropriate, I transformed continuous data to achieve normality and used parametric tests. To test treatment groups while controlling for male body mass, I conducted a two-way ANOVA. I blocked female tested and food treatment groups, using male body

mass as a covariate (Sokal and Rolf 1995) after transforming the number of visits (square root) and total time (logarithmic) data to meet normality assumptions.

Results

Both of the behavioural criteria indicated that females preferred the odours of Ad Lib males to those of Low Food males. Twelve of the 16 females spent more time in the cylinder associated with Ad Lib males (sign test: $P = 0.04$) and twelve of 15 females visited the Ad Lib male more frequently (sign test: $P = 0.02$ with one tie). Additionally, female mean number of visits (square root transformed) to Ad Lib male cylinders was greater than number of visits to Low Food male chambers ($\overline{X}$ difference $\pm$ 95% CI $= 0.68 \pm 0.51$; $t_{15} = 2.81$, $P = 0.013$). Though females tended to spend more time (log transformed) in Ad Lib male cylinders, the difference was not significant (0.17 ± 0.30 s; $t_{15} = 1.25$; $P = 0.23$). Because Ad Lib males tended to be heavier than Low Food males (Table I), I re-analyzed the visitation data controlling for male body mass. When body mass was held in check, females visited Ad Lib males more often (adjusted mean square root transformed visits: Ad Lib $= 3.95 \pm 0.79$, Low Food: 3.41 ± 0.71; $F_{1,14} = 6.76$, $P = 0.02$). The number of females that spent greater amounts of time in, and more visits in one cylinder over another did not differ from random for male mass, size of the male's natal litter, or age (Table I). Though Ad Lib males were younger than Low Food males ($\overline{X}$ difference $\pm$ 95% CI: -44.13 ± 34.81 d, $t_{15} = 2.48$, $P = 0.03$), body mass (-1.48 ± 3.08 g, $t_{15} = -0.94$, $P = 0.35$) and male's natal litter sizes (0.43 ± 0.51, $t_{15} = 1.70$, $P = 0.11$) were not demonstrably different.

Discussion

Female grasshopper mice appear able to distinguish between males raised by mothers that varied in nourishment during lactation based on male odour alone. This result is similar to mate choice tests for house mice where mothers of unattractive males were undernourished during gestation (Meikle et al. 1995). The present study suggests that, at least for small mammals, poor maternal condition during latter phases of parental investment can reduce her sons' attractiveness to potential mates as well. Stress early in development appears to have a profound effect on male reproductive success.

Ad Libitum males were also younger than Low Food males, which confounds the results of this study. Age may be detectable through scent (Bell 1983). However, younger indviduals would be expected to be at a relative <u>disadvantage</u> in competition for mates in general (Weatherhead 1984; Manning 1985). Though males differed in age, both males had no prior sexual experience. This further reinforces that females were choosing males based on male quality due to mother's condition during lactation although Ad Lib males tended to be younger than their Low Food counterparts.

Factors influencing early experience, such as brood size (collared flycatchers: Gustaffson et al.1995; rodents: reviewed in Mendl 1988), exposure to adult song (reviewed in Arnold 1994) or intrauterine position of the fetus with respect to the sex of neighbouring siblings (vom Saal et al. 1983, Clark & Galef 1995) can affect later sexual attractiveness and/or competitive ability. For instance, male fetuses located between two females in a uterine horn can, through exposure to female hormones, demasculinize the male, reducing his opportunities to mate as an adult (reviewed in Clark & Galef 1995). A reduction in resources during a male rodent's

92

dependence on milk seems to also reduce reproductive opportunities. While under-nutrition during gestation or lactation appears to jeopardize the attractiveness of sons, it is unknown how much variance occurs in the condition and attractiveness of males in the wild.

Though sexual attractiveness of male odour has been established for house mice (Blaustein 1981; Drickamer 1992; Hurst & Rich in press), it has been less established if grasshopper mice also use olfactory cues. Ruffer (1965) reported that grasshopper mice preferred odours from the opposite sex over those from same-sex individuals or no scent at all. In this genus, both sexes possess a ventral gland that functions only when circulating testosterone is present and estrogen absent (McCarty 1978). It is possible that the ventral gland plays a role in revealing male quality, though further exploration of this and other glands that potentially affect female mate choice is needed.

During lactation, growth of both female and male mouse pups is entirely dependent on milk intake (e.g., Fuchs 1982), as no other food is ingested. Due to sexual differentiation during development, male growth may be affected more acutely during the nursing period if food to the mother is in short supply (McClure 1981) or as litter size increases (Gosling et al. 1984). In such ecological squeezes, mortality may be biased towards males (McClure 1981; Moses, et al. 1998). For wild house mice, mass differences among males determined breeding access, while female mass was unrelated to reproductive success (Krackow 1993). Though food-restricted male grasshopper mice are lower in mass at weaning than Ad Lib conspecifics (Sikes 1996; Moodie 1999), this difference did not continue into adulthood. However, Low Food males that were unable to grow at rates comparable to Ad Lib males during lactation did, on average, suffer lower sexual attractiveness scores, indicating that nourishment during

lactation may influence later mating success through some as-yet-unidentified olfactory cue. While Low Food males may be able to compensate for initial growth handicaps post-weaning, the process appears to be an imperfect one. If male quality can be assessed reliably through phermones, males that overcome initial growth handicaps may not be able to mask chemical signals. For house mice and aboriginal mice (M. spretus), male dominance and sexual attractiveness is related to the same phermones produced by males (Jeniolo et al. 1985, Hurst 1990a, b). Inferior quality males thus suffer from decreased opportunities to secure resources through male-male competition and reduced sexual attractiveness.

Variation among male condition can also occur within litters (Moodie 1999). It would be interesting to determine the degree to which male attractiveness differs within the same litter. Sibling competition may allow some males to out-compete their male sibs, thereby increasing their own reproductive success. Mate choice tests and/or male competitive tests for full siblings have yet to be assessed. These tests, however, would examine the premise that sibling rivalry is an important factor in determining individual fitness for species where brood size is greater than one.

Acknowledgments

References

Allen, E. 1922. The oestrous cycle in the mouse. American Journal of Anatomy, 30, 297-371.

Arnold, A. P. 1994. Critical events in the development of bird song: what can neurobiology contribute to the study of the evolution of behavior? In: Behavioral mechanisms in evolutionary ecology (Ed. by L. A. Real), pp. 219-237. Chicago University Press.

Bell, D. J. 1983. Mate choice in the European rabbit. In: Mate Choice (Ed. by P. Bateson), pp. 211-223. New York: Cambridge University Press.

Blaustein, A. R. 1981. Sexual selection and mammalian olfaction. American Naturalist, 117, 1006-1010.

Clark, M. M. & Galef, B. G., Jr. 1995. Prenatal influences on reproductive life history strategies. Trends in Ecology and Evolution, 10, 151-153.

Clutton-Brock, T. H.,. Albon, S. D., & Guinness, F. E. 1984. Maternal dominance, breeding success, and birth sex ratios in red deer. Nature 308: 358-360.

Clutton-Brock, T. H., Guinness, F. E. & Albon, S. D. 1982. Red deer: behavior and ecology of two sexes. University of Chicago Press.

Clutton-Brock, T. H. 1988. Reproductive success. In: Reproductive Success (Ed. by T. H. Clutton-Brock), pp 472-486. University of Chicago Press.

Drickamer, L. C. 1992. Oestrous female house mice discriminate dominant from subordinate males and sons of dominant from sons of subordinate males by odour cues. Animal Behaviour, 43, 868-870.

Fuchs, S. 1982. Optimality of parental investment: the influence of nursing on reproductive success of mother and female young house mice. Behavioral Ecology and Sociobiology, 10, 39-51.

Galler, J. R., & Turkewitz, G. 1975. Variability of the effects of rearing in a large litter on the development of the rat. Developmental Psychobiology, 8, 325-331.

Gosling, L. M., Baker, S. J. & Wright, K. M. H. 1984. Differential investment by female coypus (Myocastor coypus) during lactation. In: Physiolological Strategies in Lactation (Ed. by M. Peaker, R.G. Vernon & C. H. Knight), pp. 273-300. London: Academic Press.

Gustafsson, L., Qvarnström, A. & Sheldon, B. C. 1995. Trade-offs between life-history traits and a secondary sexual character in male collared flycatchers. Nature, 375, 311-313.

Homer, B. E., & Taylor, J. M. 1968. Growth and reproductive behavior in the southern grasshopper mouse. Journal of Mammalogy, 49, 644-660.

Huck, U. W. & Banks, E. M. 1982. Differential attraction of females to dominant males: olfactory discrimination and mating preference in the brown lemming (Lemmus trimucronatus). Behavioral Ecology and Sociobiology, 11, 217-222.

Hurst, J. L. 1990a. Urine marking in populations of wild house mice Mus domesticus Rutty. I. Communication between males. Animal Behaviour, 40, 209-222.

Hurst, J. L. 1990b. Urine marking in populations of wild house mice Mus domesticus Rutty. III. Communication between the sexes. Animal Behaviour, 40, 233-243.

Hurst, J. L. & Rich, T. J. in press. Scent marks as competitive signals of mate quality. In: Chemical Signals in Vertebrates. Vol. 8 (Ed. by R. E. Johnston & D. Müller-Schwarze). New York: Plenum.

Jeniolo, B., Alberts, J., Sochinski-Wiggins, S., Harvey, S. & Novotny, M. 1985. Behavioural and endocrine responses of female mice to synthetic analogues of volatile compounds in male urine. Animal Behaviour, 33, 1114-1118.

König, B., Riester, J., & Markl, H. 1988. Maternal care in house mice (Mus musculus): II. The energy cost of lactation as a function of litter size. Journal of Zoology, London, 216, 195-210.

Krackow, S. 1993. The effect of weaning weight on offspring fitness in wild house mice (Mus musculus domesticus): a preliminary study. Ethology, 95, 76-82.

Labov, J. B., Huck, U. W., Vaswani, P. & Lisk, R. D. 1986. Sex ratio manipulation and decreased growth of male offspring of undernourished golden hamsters (Mesocricetus auratus). Behavioral Ecology and Sociobiology, 18, 241-249.

Lenington. S. 1983. Social preferences for partners carrying 'good genes' in wild house mice. Animal Behaviour, 31, 325-333.

Manning, J.T. 1985. Choosy females and correlates of male age. Journal of theoretical Biology, 108, 215-220.

McCarty, R. 1978. Onychomys leucogaster. Mammalian Species, 87, 1-6.

McClure, P. A. 1981. Sex-biased litter reduction in food-restricted wood rats (Neotoma floridana). Science, 211, 1058-1060.

Meikle, D. B., Kruper, J. H. & Browning, C. R. 1995. Adult male house mice born to undernourished mothers are unattractive to oestrous females. Animal Behaviour, 50, 753-758.

Meikle, D. B. & Vessey, S. H. 1988. Maternal dominance rank and lifetime survivorship of male and female rhesus monkeys. Behavioral Ecology and Sociobiology, 22, 379-383.

Mendl, M. 1988. The effects of litter size variation on mother-offspring relationships and behavioural and physical development in several mammalian species (principally rodents). Journal of Zoology, London, 215, 15-34.

Mendl, M., & Paul, E. S. 1989. Observation of nursing and suckling behaviour as an indicator of milk transfer and parental investment. Animal Behaviour, 37, 513-515.

Moodie, J. D. 1999. Sibling rivalry for milk in northern grasshopper mice (Onychomys leucogaster). Ph.D. thesis, University of Oklahoma, Norman.

Moses, R. A., Boutin, S. & Teferi, T. 1998. Sex-biased mortality in woodrats occurs in the absence of parental intervention. Animal Behaviour, 55, 563-571.

Paul, A., Kuester, J. & Arnemann, J. 1992. Maternal rank affects reproductive success of male Barbary macaques (Macaca sylvanus): evidence from DNA fingerprinting. Behavioral Ecology and Sociobiology, 30, 337-341.

Pinter, A. J. 1970. Reproduction and growth for two species of grasshopper mice (Onychomys) in the laboratory. Journal of Mammalogy, 51, 236-243.

Ruffer, D. G. 1965. Sexual behaviour of the northern grasshopper mouse (Onychomys leucogaster). Animal Behaviour, 13, 447-452.

Sikes, R. S. 1995. Costs of lactation and optimal litter size in northern grasshopper mice (Onychomys leucogaster). Journal of Mammalogy, 76, 348-357.

Sikes, R. S. 1996. Effects of maternal nutrition on post-weaning growth in two North American rodents. Behavioral Ecology and Sociobiology, 38, 303-310.

Thomas, K. J., & Dominic, C. J. 1988. Individual recognition of the stud male by female mice: role of pheromones and of the olfactory-vomeronasal systems. Biology of Behaviour, 13, 164-174.

Thompson, S. D. 1992. Gestation and lactation in small mammals: basal metabolic rate and the limits of energy use. In: Mammalian Energetics: interdisciplinary views of metabolism and reproduction (Ed. by T. E. Tomasi & T. H. Horton) pp. 213-259. Ithaca, New York: Cornell University Press.

Trivers, R. L., & Willard, D. E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. Science, 179, 90-92.

vom Saal, F. S., Grant, W. M., McMullen, C. W. & Laves, K. S. 1983. High fetal estrogen concentrations: correlation with increased adult sexual activity and decreased aggression in male mice. Science, 220, 1306-1309.

Weatherhead, P. J. 1984. Mate choice in avian polygyny: why do females prefer older males? American Naturalist, 123, 873-875.

Wright, S. L., Crawford, C. B. & Anderson, J. L. 1988. Allocation of reproductive effort in Mus domesticus: responses of offspring sex ratio and quality to social density and food availability. Behavioral Ecology and Sociobiology, 23, 357-365.
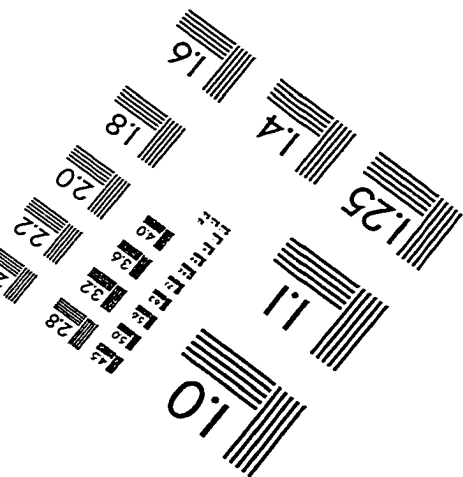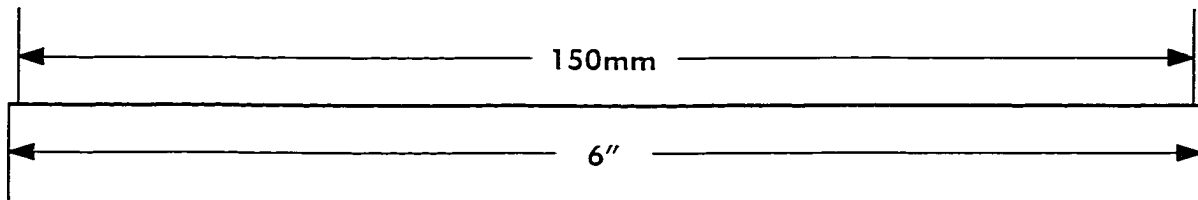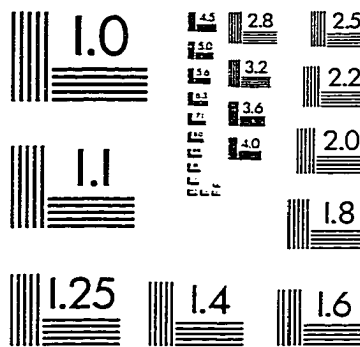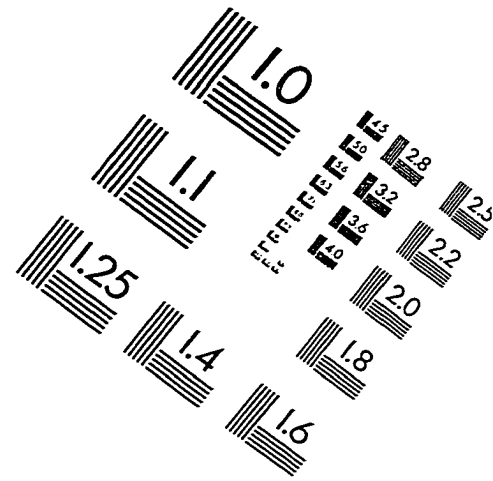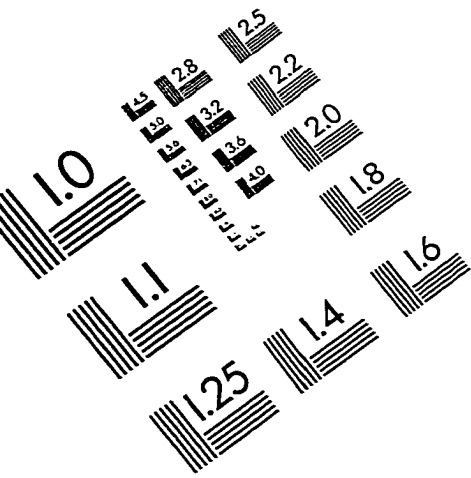
Table I

Preferences of oestrous female grasshopper mice (N = 16) for odours of males whose mothers were fed ad libitum (ADLIB males) versus males whose mothers were food-restricted (LOFOOD males) during the first 24 days post-partum.

	Number of trials female spent more time with	Number of trials female visited more often with
Son of Ad Libitum female	12*	12†
Son of Restricted female	4	3
Mass: male Heavy	10	11
Mass: male Light	6	4
Litter Size: from Large litter	6	6
Litter Size: from Small litter	4	3
Age: male Older	5	6
Age: male Younger	11	9

Sum of number of trials between two categories less than 16 due to ties.

Sign test used for all comparisons; * $\underline{P}$ = 0.04; † $\underline{P}$ = 0.02.

IMAGE EVALUATION
TEST TARGET (QA-3)

150mm

6"