Title

Intrinsic and Social Influences on Family Dynamics and Offspring Development in a Biparental Rodent

Author

Nguyen, Catherine T.Y.

Publication Date

2023

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Intrinsic and Social Influences on Family Dynamics and Offspring Development in a Biparental Rodent

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Thien-Y Catherine Nguyen

December 2023

Dissertation Committee:
    Dr. Wendy Saltzman, Chairperson
    Dr. Polly Campbell
    Dr. Christopher Clark

The Dissertation of Thien-Y Catherine Nguyen is approved:

Committee Chairperson

University of California, Riverside

Acknowledgements

I thank Dr. Wendy Saltzman for her guidance and support over the course of the last six years. Through her, I have learned how to be a good scientist and mentor to others.

I would like to express my gratitude to my labmates, Melina Acosta and April Arquilla. As colleagues and friends, they have provided so much joy and comfort that have led me to become the person and scientist that I am today. We started together as graduate students in the Saltzman lab at the same time, and I hope we continue our journeys in parallel for years to come. My friends, you bow to no one. I also thank all former and current undergraduate and postbaccalaureate researchers of the Saltzman lab. This dissertation would have been impossible without their contributions to animal maintenance and data collection. I give my greatest thanks to their enthusiasm and curiosity about science for keeping me motivated while in the depths of research. I also thank Johnny Pham and the rest of the Spieth Vivarium staff for animal care and Laurie Graham for all the help with creating and maintaining research equipment. Lastly, I thank all staff members at UCR, including EMM, Lab Prep, custodial services, and EH&S. No research at UCR would be possible without our non-academic staff.

I would like to thank my dissertation and oral exam committee members, Drs. Polly Campbell, Chris Clark, Kim Hammond, Hollis Woodard, and Misaki Natsuaki, for their encouragement, mentorship, and support given the challenges of my oral exam. I especially thank Polly for her leadership as chair of my oral exam committee and, most of

all, for her kindness and empathy which I will never forget. I would also like to thank Dr. Ted Garland, Dr. Alison Greggor, Elijah Hall, Annika Rose-Person, Chris Cosma, and Jeff Ellis for all of their help with statistical analyses in this dissertation.

Words cannot describe the love and gratitude that I have for all of the friends I've made. I have had so many people who showed kindness and friendship through the hardest moments of my life. I especially thank Paul Nguyen (my best friend), Kelly Trinh, Linda Banh, Steven Nguyen, Peter Truong, and Cindy Nguyen for all their continued support, joy, and friendship ever since elementary and high school. They have been there since the beginning of my interest in science and animal behavior, and I cherish the deep bonds we have. I also particularly express my gratitude to April Arquilla, Melina Acosta, Ayala Berger, Sarah Gardner, Sandhya Sriram, William Ota, Elijah Hall, Annika Rose-Person, Chris Cosma, and Andrea Keller for all of the friendships, laughs, comfort, and adventures we've had together, whether in reality or worlds fantastical. As difficult as my time as a graduate student was, I would go through it again just to relive our time together. I would also like to thank Jess Macarro, Joshua Reger, Jeff Ellis, and all members of SciComm@UCR. I have had such rewarding experiences and befriended incredible people who share my passion for science communication and increasing accessibility to learning science.

Thank you to the NSF Graduate Research Fellowship program, the Society of Integrative and Comparative Biology, and UCR Graduate Division for funding my research and studies. I also thank the NSF, Dr. Debra Shier, and the San Diego Zoo

Wildlife Alliance for an unbelievable internship that allowed me to enter the world of conservation which has shaped me into a better scientist and person.

I would like to thank my dad for encouraging a lifelong love of science and all of his support during the last six years. I would not be a scientist without him. Finally, I thank Persephone, my beloved crested gecko, and all of the pets that I have had the pleasure of meeting.

Dedication

To my father and all of the times we went to look at ducks and geese at the park. That’s where it all started.

ABSTRACT OF THE DISSERTATION

Intrinsic and Social Influences on Family Dynamics and Offspring Consequences in a Biparental Rodent

by

Thien-Y Catherine Nguyen

Doctor of Philosophy, Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2023
Dr. Wendy Saltzman, Chairperson

In many species, caring for young is essential for reproductive success because it increases offspring survival, development, and quality. However, parental care can be costly by increasing resource expenditure and mortality risk and decreasing likelihood of future reproduction. Therefore, animals are predicted to adjust their levels of caregiving based on both intrinsic (e.g., sex, physical ability) and extrinsic factors (e.g., season, social interactions). Within-family dynamics are particularly interesting because an animal's level of care determined by intrinsic factors may be influenced by responses to the behaviors of mates, offspring, or siblings. In biparental species, an individual's social environment includes not only offspring, which can manipulate parents to garner greater levels of care, but also its mate. Little is known about how interactions within biparental families affect parental behavior. Thus, I examined intrinsic and social influences on caregiving behavior and the consequences for offspring in a biparental mammal, the California mouse (*Peromyscus californicus*). The first study investigated sex as a factor by determining whether parent-offspring discrimination differs between mothers and fathers. Throughout the pup-rearing period, I presented individual parents simultaneously with two mesh balls containing their own pup in one and an unrelated pup in the other. Although parents behaved similarly on individual test days, fathers showed significant changes in behaviors directed towards the non-kin ball over time, suggesting that fathers may show increased interest in unrelated young as their offspring become independent. In the second study, I examined parental behavior of both parents and offspring quality are affected by paternal age. Mothers mated to young fathers nursed pups more than those mated to old fathers; however, few other measures differed between families of old and young fathers. In the last study, I explored the effects of overlapping litters, in which subsequent litters are raised concurrently, on parent-offspring and sibling-sibling interactions and on offspring development.

Table of Contents

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

Introduction

Parental investment has been defined as any investment in current offspring that reduces an individual's ability to invest in future offspring (Trivers, 1972). One form of parental investment is through parental care, which comprises behaviors and physiological processes that increase offspring fitness (Clutton-Brock, 1991). Parental care is found across many taxa, including insects, birds, reptiles, amphibians, and mammals, and can range from brooding eggs to behaviors directed at offspring after birth or hatching (Clutton-Brock, 1991; P. T. Smith, Kolliker, & Royle, 2012). Although parental care can improve the parent's fitness, providing care can lead to increased need for resources and risk of injury or death. Whether or not parental care evolves depends on life history, environmental factors, capability to provide care, fitness benefits, and the ability of a genetic mutation promoting parental care to spread in a population (P. T. Smith et al., 2012).

Biparental care systems, in which both mothers and fathers provide care to offspring, can reduce individual costs of rearing young, which may increase the parents' lifetime reproductive success (Pilakouta, Hanlon, & Smiseth, 2018; P. T. Smith et al., 2012; Woodroffe & Vincent, 1994) and, in some species, is necessary for offspring survival (Wittenberger & Tilson, 1980). In mammals, mothers must gestate and lactate, which eliminates the need for incubation and immediate food provisioning for offspring (Storey & Walsh, 2013). Consequently, paternal care (i.e., care of offspring by fathers) is less common than maternal care (i.e., care of offspring by mothers): only approximately 5-10% of mammalian species display paternal behavior (Kleiman & Malcolm 1981).

Aside from parental care, alloparental care refers to care provided to non-descendant young. Kin-selection theory predicts that individuals should behave altruistically according to their relatedness to the recipient (Hamilton, 1964). Consistent with this prediction, alloparents are often related to the young that they care for, such as siblings. In addition to inclusive fitness, benefits of being an alloparent can include gaining experience in caregiving behavior, increasing attractiveness to prospective mates, reciprocal altruism, and, in primates, exploitation of infants for social benefits (Riedman, 1982; Stiver & Alonzo, 2011).

Both intrinsic qualities of an individual and its environment can influence the amount and quality of care it provides to young. Intrinsic factors include physical condition and neuroendocrine mechanisms underlying parental and alloparental behavior. Sex (Caldwell, 2018; Jurkevich, Grossmann, Balthazart, & Viglietti-Panzica, 2001; Lonstein & De Vries, 2000), age (Angelier, Shaffer, Weimerskirch, & Chastel, 2006; Ottinger, 2010; Rogers, Rhemtulla, Ferrer, & Bales, 2018) and life history stage (Bridges, 2016; Ziegler, 2000) may influence neuroendocrine processes, leading to variation in caregiving behavior across a population. Extrinsic factors that can influence caregiving include both abiotic (e.g., seasonal changes) and biotic (e.g., predation, food availability, social environment) variables.

In this dissertation, I focus on interactions with conspecifics as an external factor affecting care. Parents and alloparents are predicted to adjust their caregiving according to the levels of care provided by other caregivers (Clutton-Brock, 1991; Harrison, Barta, Cuthill, & Székely, 2009; Johnstone & Hinde, 2006). In turn, offspring can influence the amount of care they receive through cues, such as begging behavior and distress vocalizations, to indicate their needs. However, offspring may exploit these cues to receive additional care, leading to parent-offspring conflict (Trivers, 1974). Additionally, parents may manipulate older juveniles into remaining near the natal site and providing alloparental care to younger siblings (Trivers, 1974).

Through caregiving behaviors, parents and alloparents can profoundly shape the physical and behavioral development of young. The internal and external environments can affect one another through early-life effects. In particular, the amount of care an infant receives can influence its neuroendocrine development and epigenome, leading to differential parental, exploratory, and anxiety-like behaviors in adulthood (Braun & Champagne, 2014; Caldji, Diorio, & Meaney, 2000; Champagne, 2008). Additionally, early-life stress can have profound effects on development and adult behavior (Bolton, Molet, Ivy, & Baram, 2017; Murgatroyd, Peña, Podda, Nestler, & Nephew, 2015). Thus, the interactions between intrinsic and extrinsic factors can have transgenerational effects on behavior and reproductive success.

In mammals, many studies have focused on the presence of caregivers or manipulations of caregivers by the experimenters, leaving the role of spontaneously occurring social interactions relatively unexplored. Furthermore, studies that do examine the effects of social dynamics on caregiving have mostly been conducted in highly social mammals, such as primates and meerkats (Ahern, Hammock, & Young, 2010; English, 2009; Harper, 1981; Rogers et al., 2018; Swartz & Rosenblum, 1981). This raises the question of how family dynamics influence caregiving in mammals that live in smaller groups where, presumably, the only conspecifics that an infant encounters are its parents and siblings.

I used the California mouse as a model to identify variables that affect family dynamics (parent-offspring, parent-parent, and sibling-sibling interactions) and how these variables influence behavior and offspring outcomes in biparental mammals. California mice are socially monogamous both in the field and in the lab, and live in small family groups consisting of a mated pair and their offspring (Ribble & Salvioni, 1990). They are found in chaparral and woodlands along the coast from San Francisco to Baja California (McCabe & Blanchard, 1950; Merritt, 1978). Pairs breed throughout the year in the wild, with a birth peak in the winter, and litters typically comprise one to four pups (McCabe & Blanchard, 1950; Ribble, 1992b). Fathers display the same parental behaviors as mothers, with the exception of nursing (Gubernick & Alberts, 1987). Parental care has been studied extensively in the California mouse (Bales & Saltzman, 2016; Bester-Meredith, Burns, Conley, Mammarella, & Ng, 2017), but the intrinsic and extrinsic factors that influence care are understudied.

My dissertation aims to provide insight into how various qualities of parents and offspring shape family dynamics, leading to potential consequences for offspring, in a biparental system. First, I investigate sex differences in parent-offspring discrimination between mothers and fathers. Then, I explore the effects of paternal age on biparental dynamics and offspring quality. Finally, I examine how raising overlapping litters affects parent-offspring and sibling-sibling interactions as well as offspring physiological and behavioral development. Together, these studies contribute to our understanding of how intrinsic and social factors affect parental care and reproductive success in mammals.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2010). Parental Division of Labor , Coordination , and the Effects of Family Structure on Parenting in Monogamous Prairie Voles (Microtus ochrogaster). https://doi.org/10.1002/dev.20498

Angelier, F., Shaffer, S. A., Weimerskirch, H., & Chastel, O. (2006). Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird: The wandering albatross. *General and Comparative Endocrinology*, *149*(1), 1–9. https://doi.org/10.1016/j.ygcen.2006.04.006

Angell, C. S., Janacek, R., & Rundle, H. D. (2022). Maternal and Paternal Age Effects on Male Antler Flies: A Field Experiment. *American Naturalist*, *199*(3), 436–442. https://doi.org/10.1086/718236

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology*, *14*(4), 451–479.

Auroux, M. (1983). Decrease of learning capacity in offspring with increasing paternal age in the rat. *Teratology*, *27*(2), 141–148. https://doi.org/10.1002/tera.1420270202

Bales, K. L., & Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Hormones and Behavior*, *77*, 249–259. https://doi.org/10.1016/j.yhbeh.2015.05.021

Benowitz, K. M., Head, M. L., Williams, C. A., Moore, A. J., & Royle, N. J. (2013). Male age mediates reproductive investment and response to paternity assurance. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1764), 20131124. https://doi.org/10.1098/rspb.2013.1124

Bester-Meredith, J. K., Burns, J. N., Conley, M. F., Mammarella, G. E., & Ng, N. D. (2017). Peromyscus as a model system for understanding the regulation of maternal behavior. *Seminars in Cell and Developmental Biology*, *61*, 99–106. https://doi.org/10.1016/j.semcdb.2016.07.001

Birnie, A. K., Taylor, J. H., Cavanaugh, J., & French, J. A. (2013). Quality of maternal and paternal care predicts later stress reactivity in the cooperatively-breeding marmoset (Callithrix geoffroyi). *Psychoneuroendocrinology*, *38*(12), 3003–3014. https://doi.org/10.1016/j.psyneuen.2013.08.011

Boheemen, L. A. Van, Richardson, D. S., Burke, T., Komdeur, J., & Dugdale, H. L. (2019). Compensatory and additive helper effects in the cooperatively breeding Seychelles warbler (Acrocephalus sechellensis), (October 2018), 2986–2995. https://doi.org/10.1002/ece3.4982

Bolton, J. L., Molet, J., Ivy, A., & Baram, T. Z. (2017). New insights into early-life stress and behavioral outcomes. *Current Opinion in Behavioral Sciences*, *14*, 133–139. https://doi.org/10.1016/j.cobeha.2016.12.012

Braun, K., & Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *Journal of Neuroendocrinology*, *26*(10), 697–706. https://doi.org/10.1111/jne.12174

Bridges, R. S. (2016). Long-term alterations in neural and endocrine processes induced by motherhood in mammals. *Hormones and Behavior*, *77*, 193–203. https://doi.org/10.1016/j.yhbeh.2015.09.001

Brooks, M. E., Kristensen, K., Benthem, K. J. van, Magnusson, A., Berg, C. W., Nielsen, A., … Bolker, B. M. (2017). Modeling zero-inflated count data with glmmTMB. *BioRxiv*, 132753. Retrieved from https://www.biorxiv.org/content/10.1101/132753v1%0Ahttps://www.biorxiv.org/content/10.1101/132753v1.abstract

Burley, N. (1988). The differential-allocation hypothesis: an experimental test. *American Naturalist*, *132*(5), 611–628. https://doi.org/10.1086/284877

Caballero-Campo, P., Lin, W., Simbulan, R., Liu, X., Feuer, S., Donjacour, A., & Rinaudo, P. F. (2018). Advanced Paternal Age Affects Sperm Count and Anogenital Distance in Mouse Offspring. *Reproductive Sciences*, *25*(4), 515–522. https://doi.org/10.1177/1933719118759441

Caldji, C., Diorio, J., & Meaney, M. J. (2000). Variations in maternal care in infancy regulate the development of stress reactivity. *Biological Psychiatry*, *48*(12), 1164–1174. https://doi.org/10.1016/S0006-3223(00)01084-2

Caldwell, H. K. (2018). Oxytocin and sex differences in behavior. *Current Opinion in Behavioral Sciences*, *23*, 13–20. https://doi.org/10.1016/j.cobeha.2018.02.002

Cameron, E. Z. (1998). Is suckling behavior a useful predictor of milk intake? A review. *Animal Behaviour*, *56*(3), 521–532. https://doi.org/10.1006/anbe.1998.0793

Cameron, E. Z., Linklater, W. L., Stafford, K. J., Minot, E. O. 0, Minot, E. O. 0, Cameron, E. Z., … Minot, E. O. 0. (2000). Aging and Improving Reproductive Success in Horses: Declining Residual Reproductive Value or Just Older and Wiser? *Source: Behavioral Ecology and Sociobiology Behav Ecol Sociobiol*, *47*(47), 243–249. Retrieved from http://www.jstor.org/stable/4601737

Cantoni, D., Glaizot, O., & Brown, R. E. (1999). Effects of sex composition of the litter on anogenital distance in California mice (Peromyscus californicus). *Canadian Journal of Zoology*, *77*(1), 124–131. https://doi.org/10.1139/cjz-77-1-124

Carrageta, D. F., Guerra-carvalho, B., Angélica, M., Marc, S., Oliveira, P. F., & Alves, M. G. (2022). Animal models of male reproductive ageing to study testosterone production and spermatogenesis, 1341–1360.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, *29*(3), 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Cholewa, M., Jankowiak, Ł., Szenejko, M., Dybus, A., Śmietana, P., & Wysocki, D. (2021). The effects of parental age difference on the offspring sex and fitness of European blackbirds. *PeerJ*, *9*, 1–20. https://doi.org/10.7717/peerj.10858

Clark, M. M., Moghaddas, M., & Galef, B. G. (2002). Age at first mating affects parental effort and fecundity of female Mongolian gerbils. *Animal Behaviour*, *63*(6), 1129–1134. https://doi.org/10.1006/anbe.2002.3019

Clutton-brock, T. H. (2004). Cooperative breeding in mammals.

Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, New Jersey, USA: Princeton University Press.

Creel, S., Dantzer, B., Goymann, W., & Rubenstein, D. R. (2013). The ecology of stress: Effects of the social environment. *Functional Ecology*, *27*(1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x

Crick, H. Q. P. (1992). Load-lightening in cooperatively breeding birds and the cost of reproduction. *Ibis*, *134*(1), 56–61. https://doi.org/10.1111/j.1474-919X.1992.tb07230.x

Dudley, D. (1974). Contributions of paternal care to the growth and development of the young in Peromyscus californicus. *Behavioral Biology*, *11*(2), 155–166. https://doi.org/10.1016/S0091-6773(74)90305-8

English, S. (2009). Individual variation in cooperative behaviour in meerkats, (October).

Ericsson, G., Wallin, K., Ball, J. P., & Broberg, M. (2001). Age-related reproductive effort and senescence in free-ranging moose, Alces alces. *Ecology*, *82*(6), 1613–1620. https://doi.org/10.1890/0012-9658(2001)082[1613:ARREAS]2.0.CO;2

Fay, R., Barbraud, C., Delord, K., & Weimerskirch, H. (2016). Paternal but not maternal age influences early-life performance of offspring in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828). https://doi.org/10.1098/rspb.2015.2318

Fisher, R. A. (1930). *The genetical theory of natural selection. The genetical theory of natural selection.* Oxford, England: Clarendon Press. https://doi.org/10.5962/bhl.title.27468

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Sage publications.

Francis, D. D., Champagne, F. A., Liu, D., & Meaney, M. J. (1999). Maternal care, gene expression, and the development of individual differences in stress reactivity. *Annals of the New York Academy of Sciences*, *896*, 66–84. https://doi.org/10.1111/j.1749-6632.1999.tb08106.x

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

García-Palomares, S., Navarro, S., Pertusa, J. F., Hermenegildo, C., García-Pérez, M. A., Rausell, F., … Tarín, J. J. (2009). Delayed Fatherhood in Mice Decreases Reproductive Fitness and Longevity of Offspring. *Biology of Reproduction*, *80*(2), 343–349. https://doi.org/10.1095/biolreprod.108.073395

García-Palomares, S., Pertusa, J. F., Miñarro, J., García-Pérez, M. A., Hermenegildo, C., Rausell, F., … Tarín, J. J. (2009). Long-Term Effects of Delayed Fatherhood in Mice on Postnatal Development and Behavioral Traits of Offspring. *Biology of Reproduction*, *80*(2), 337–342. https://doi.org/10.1095/biolreprod.108.072066

Gilbert, A. N. (1995). Tenacious nipple attachment in rodents: the sibling competition hypothesis. *Animal Behaviour*, *50*(4), 881–891. https://doi.org/10.1016/0003-3472(95)80091-3

González-Mariscal, G., Caba, M., Martínez-Gómez, M., Bautista, A., & Hudson, R. (2016). Mothers and offspring: The rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. *Hormones and Behavior*, *77*, 30–41. https://doi.org/10.1016/j.yhbeh.2015.05.011

Greenberg, G. D., van Westerhuyzen, J. A., Bales, K. L., & Trainor, B. C. (2012). Is it all in the family? The effects of early social structure on neural-behavioral systems of prairie voles (Microtus ochrogaster). *Neuroscience*, *216*, 46–56. https://doi.org/10.1016/j.neuroscience.2012.04.063

Gromov, V. S. (2009). Interactions of partners in family pairs, care of the offspring, and the role of tactile stimulation in formation of parental behavior of the Mongolian gerbil (Meriones unguiculatus) under laboratory conditions. *Biology Bulletin*, *36*(5), 479–488. https://doi.org/10.1134/s1062359009050082

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. https://doi.org/10.2307/1381649

Gubernick, D. J., & Addington, R. L. (1994). The stability of female social and mating preferences in the monogamous California mouse, peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1079

Gubernick, D. J., & Alberts, J. R. (1987). The biparental care system of the California mouse, Peromyscus californicus.. *Journal of Comparative Psychology*, *101*(2), 169–177. https://doi.org/10.1037/0735-7036.101.2.169

Gubernick, D. J., & Laskin, B. (1994). Mechanisms influencing sibling care in the monogamous biparental California mouse, Peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1356

Gubernick, D. J., & Nordby, J. C. (1992). Parental influences on female puberty in the monogamous California mouse, Peromyscus californicus. *Animal Behaviour*, *44*(PART 2), 259–267. https://doi.org/10.1016/0003-3472(92)90032-5

Gubernick, D. J., & Nordby, J. C. (1993). Mechanisms of sexual fidelity in the monogamous California mouse, Peromyscus californicus. *Behavioral Ecology and Sociobiology*, *32*(3), 211–219. https://doi.org/10.1007/BF00173779

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *The Royal Society*, *267*(1439), 147–150. https://doi.org/10.1098/rspb.2000.0979

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Hardy, M. P., & Schlegel, P. N. (2004). Testosterone Production in the Aging Male: Where Does the Slowdown Occur? *Endocrinology*, *145*(10), 4439–4440. https://doi.org/10.1210/en.2004-0888

Harper, L. V. (1981). Offspring effects upon parents. In *Parental care in mammals* (pp. 117–177). Springer.

Harris, B. N., & Saltzman, W. (2013). Effects of aging on hypothalamic-pituitary-adrenal (HPA) axis activity and reactivity in virgin male and female California mice (Peromyscus californicus). *General and Comparative Endocrinology*, *186*, 41–49. https://doi.org/10.1016/j.ygcen.2013.02.010

Harrison, F., Barta, Z., Cuthill, I., & Székely, T. (2009). How is sexual conflict over parental care resolved? A meta-analysis. *Journal of Evolutionary Biology*, *22*(9), 1800–1812. https://doi.org/10.1111/j.1420-9101.2009.01792.x

Hartig, F., & Hartig, M. F. (2017). Package ‘DHARMa.’ *R Package*.

Havighorst, A., Crossland, J., & Kiaris, H. (2017). Peromyscus as a model of human disease. *Seminars in Cell and Developmental Biology*, *61*, 150–155. https://doi.org/10.1016/j.semcdb.2016.06.020

Heinsohn, R., & Legge, S. (1999). The cost of helping. *Trends in Ecology and Evolution*, *14*(2), 53–57. https://doi.org/10.1016/S0169-5347(98)01545-6

Houslay, T. M., Kitchener, P. A., & Royle, N. J. (2020). Are older parents less flexible? Testing age-dependent plasticity in Nicrophorus vespilloides burying beetles. *Animal Behaviour*, *162*, 79–86. https://doi.org/https://doi.org/10.1016/j.anbehav.2020.01.012

Houston, A. I., Székely, T., & McNamara, J. M. (2005). Conflict between parents over care. *Trends in Ecology and Evolution*, *20*(1), 33–38. https://doi.org/10.1016/j.tree.2004.10.008

Hudson, R., Bautista, A., Reyes-Meza, V., Montor, J. M., & Rödel, H. G. (2011). The effect of siblings on early development: A potential contributor to personality differences in mammals. *Developmental Psychobiology*, *53*(6), 564–574. https://doi.org/10.1002/dev.20535

Hudson, R., & Trillmich, F. (2008). Sibling competition and cooperation in mammals: Challenges, developments and prospects. *Behavioral Ecology and Sociobiology*, *62*(3), 299–307. https://doi.org/10.1007/s00265-007-0417-z

Jašarević, E., Williams, S. A., Roberts, R. M., Geary, D. C., & Rosenfeld, C. S. (2012). Spatial navigation strategies in Peromyscus: A comparative study. *Animal Behaviour*, *84*(5), 1141–1149. https://doi.org/10.1016/j.anbehav.2012.08.015

Jia, R., Tai, F., An, S., & Zhang, X. (2011). Neonatal paternal deprivation or early deprivation reduces adult parental behavior and central estrogen receptor α expression in mandarin voles (Microtus mandarinus). *Behavioural Brain Research*, *224*(2), 279–289. https://doi.org/10.1016/j.bbr.2011.05.042

Johnson, S. L., & Gemmell, N. J. (2012). Are old males still good males and can females tell the difference?: Do hidden advantages of mating with old males off-set costs related to fertility, or are we missing something else. *BioEssays*, *34*(7), 609–619. https://doi.org/10.1002/bies.201100157

Johnstone, R. A., & Hinde, C. A. (2006). Negotiation over offspring care — how should parents respond to each other’ s efforts ?, (July). https://doi.org/10.1093/beheco/arl009

Jurkevich, A., Grossmann, R., Balthazart, J., & Viglietti-Panzica, C. (2001). Gender-related changes in the avian vasotocin system during ontogeny. *Microscopy Research and Technique*, *55*(1), 27–36. https://doi.org/10.1002/jemt.1153

Kenkel, W. M., Paredes, J., Yee, J. R., Bales, K. L., & Carter, C. S. (2012). Neuroendocrine and Behavioural Responses to Exposure to an Infant in Male Prairie Voles Neuroendocrinology, (12), 874–886. https://doi.org/10.1111/j.1365-2826.2012.02301.x

Komdeur, J., & Ekman, J. (2010). Adaptations and constraints in the evolution of delayed dispersal: implications for cooperation. *Social Behaviour: Genes, Ecology and Evolution. Cambridge Univ. Press, Cambridge*, 306–327.

Kross, S. M., & Nelson, X. J. (2013). Factors influencing the behavioural development of juvenile New Zealand Falcons (Falco novaeseelandiae). *Emu*, *113*(1), 84–87. https://doi.org/10.1071/MU12020

Lecomte, V. J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., … Chastel, O. (2010). Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Sciences*, *107*(14), 6370–6375. https://doi.org/10.1073/pnas.0911181107

Lemaître, J. F., & Gaillard, J. M. (2017). Reproductive senescence: new perspectives in the wild. *Biological Reviews*, *92*(4), 2182–2199. https://doi.org/10.1111/brv.12328

Lonstein, J. S., & De Vries, G. J. (2000). Sex differences in the parental behavior of rodents. *Neuroscience and Biobehavioral Reviews*, *24*(6), 669–686. https://doi.org/10.1016/S0149-7634(00)00036-1

Mashoodh, R., Franks, B., Curley, J. P., & Champagne, F. A. (2012). Paternal social enrichment effects on maternal behavior and offspring growth. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(SUPPL.2), 17232–17238. https://doi.org/10.1073/pnas.1121083109

McCabe, T. T., & Blanchard, B. D. (1950). Three species of Peromyscus.

Merritt, J. F. (1978). Peromyscus californicus. *Mammalian Species*, (85), 1. https://doi.org/10.2307/3503909

Miyamoto, M., Kiyota, Y., Nishiyama, M., & Nagaoka, A. (1992). Senescence-Accelerated Mouse (SAM): Age-Related Reduced Anxiety-Like Behavior in the SAM-P / 8 Strain, *51*(17), 979–985.

Momand, J. R., Xu, G., & Walter, C. A. (2013). Minireview The Paternal Age Effect : A Multifaceted Phenomenon, *88*(March), 1–9. https://doi.org/10.1095/biolreprod.112.103440

Monaghan, P., Maklakov, A. A., & Metcalfe, N. B. (2020). Intergenerational Transfer of Ageing: Parental Age and Offspring Lifespan. *Trends in Ecology and Evolution*, *35*(10), 927–937. https://doi.org/10.1016/j.tree.2020.07.005

Murgatroyd, C. A., Peña, C. J., Podda, G., Nestler, E. J., & Nephew, B. C. (2015). Early life social stress induced changes in depression and anxiety associated neural pathways which are correlated with impaired maternal care. *Neuropeptides*, *52*(3), 103–111. https://doi.org/10.1016/j.npep.2015.05.002

Nguyen, C. T. Y., Zhao, M., & Saltzman, W. (2020). Effects of sex and age on parental motivation in adult virgin California mice. *Behavioural Processes*, *178*(June), 104185. https://doi.org/10.1016/j.beproc.2020.104185

Ohl, F. (2003). Testing for anxiety. *Clinical Neuroscience Research*, *3*(4–5), 233–238. https://doi.org/10.1016/S1566-2772(03)00084-7

Ottinger, M. A. (2010). Mechanisms of reproductive aging: conserved mechanisms and environmental factors. *Annals of the New York Academy of Sciences*, *1204*(1), 73–81. https://doi.org/10.1111/j.1749-6632.2010.05653.x

Perea-Rodriguez, J. P., Zhao, M., Harris, B. N., Raqueno, J., & Saltzman, W. (2018). Behavioral and endocrine consequences of placentophagia in male California mice (Peromyscus californicus). *Physiology & Behavior*, *188*(November 2017), 283–290. https://doi.org/10.1016/j.physbeh.2018.02.022

Pike, K. N., Ashton, B. J., Morgan, K. V, & Ridley, A. R. (2019). Social and Individual Factors Influence Variation in Offspring Care in the Cooperatively Breeding Western Australian Magpie, *7*(April), 1–13. https://doi.org/10.3389/fevo.2019.00092

Pilakouta, N., Hanlon, E. J. H., & Smiseth, P. T. (2018). Biparental care is more than the sum of its parts: Experimental evidence for synergistic effects on offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884), 1–7. https://doi.org/10.1098/rspb.2018.0875

Pillay, N., & Rymer, T. L. (2015). Alloparenting enhances the emotional, social and cognitive performance of female African striped mice, Rhabdomys pumilio. *Animal Behaviour*, *99*, 43–52. https://doi.org/10.1016/j.anbehav.2014.10.003

R Core Team. (2023). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Reale, D., & Bousses, P. (1995). Effect of ewe age and high population density on the early nursing behaviour of mouflon. *Ethology Ecology and Evolution*, *7*(4), 323–334. https://doi.org/10.1080/08927014.1995.9522940

Reeve, H. K. (1992). Queen activation of lazy workers in colonies of the eusocial naked mole-rat, *358*(July), 147–149.

Ribble, D. O. (1990). *Population and social dynamics of the California mouse (Peromyscus californicus)*. University of California, Berkeley.

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 161–166. https://doi.org/10.1007/BF00166397

Ribble, D. O. (1992a). Dispersal in a monogamous rodent, Peromyscus californicus. *Ecology*, *73*(3), 859–866. https://doi.org/10.2307/1940163

Ribble, D. O. (1992b). Lifetime reproductive success and its correlates in the monogamous rodent, Peromyscus californicus. *Journal of Animal Ecology*, *61*(2), 457–468. https://doi.org/10.2307/5336

Ribble, D. O., & Salvioni, M. (1990). Social Organization and Nest Co-Occupancy in Peromyscus californicus, a monogamous rodent, 9–15.

Riedman, M. L. (1982). The Evolution of Alloparental Care and Adoption in Mammals and Birds. *The Quarterly Review of Biology*, *57*(4), 405–435. https://doi.org/10.1086/412936

Robitzsch, A., Grund, S., Henke, T., & Robitzsch, M. A. (2017). Package ‘miceadds.’ *Vienna: R Foundation for Statistical Computing*.

Rödel, H. G., Meyer, S., Prager, G., Stefanski, V., & Hudson, R. (2010). Litter size is negatively correlated with corticosterone levels in weanling and juvenile laboratory rats. *Physiology and Behavior*, *99*(5), 644–650. https://doi.org/10.1016/j.physbeh.2010.01.032

Rödel, H. G., Von Holst, D., & Kraus, C. (2009). Family legacies: Short- and long-term fitness consequences of early-life conditions in female European rabbits. *Journal of Animal Ecology*, *78*(4), 789–797. https://doi.org/10.1111/j.1365-2656.2009.01537.x

Rogers, F. D., Rhemtulla, M., Ferrer, E., & Bales, K. L. (2018). Longitudinal trajectories and inter-parental dynamics of prairie vole biparental care. *Frontiers in Ecology and Evolution*, *6*(JUN), 1–13. https://doi.org/10.3389/fevo.2018.00073

Rosenbaum, S., & Gettler, L. T. (2018). Physiology & Behavior With a little help from her friends (and family) part I : the ecology and evolution of non-maternal care in mammals. *Physiology & Behavior*, *193*(November 2017), 1–11. https://doi.org/10.1016/j.physbeh.2017.12.025

Royle, N. J., Smiseth, P. T., & Kölliker, M. (2012). *The evolution of parental care*. Oxford University Press.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neuroscience and Biobehavioral Reviews*, *26*(6), 665–678. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12479841

Salo, A. L., & French, J. A. (1989). Early experience, reproductive success, and development of parental behaviour in Mongolian gerbils. *Animal Behaviour*, *38*(4), 693–702. https://doi.org/10.1016/S0003-3472(89)80015-6

Sasvári, L., Hegyi, Z., Csörgõ, T., & Hahn, I. (2000). Age-dependent diet change, parental care and reproductive cost in tawny owls Strix aluco. *Acta Oecologica*, *21*(4–5), 267–275. https://doi.org/10.1016/S1146-609X(00)01080-8

Scheibler, E., Weinandy, R., & Gattermann, R. (2005). Intra-family aggression and offspring expulsion in Mongolian gerbils (Meriones unguiculatus) under restricted environments. *Mammalian Biology*, *70*(3), 137–146. https://doi.org/10.1016/j.mambio.2004.12.002

Sheldon, B. C. (2000). Differential allocation: Tests, mechanisms and implications. *Trends in Ecology and Evolution*, *15*(10), 397–402. https://doi.org/10.1016/S0169-5347(00)01953-4

Smith, P. T., Kolliker, M., & Royle, N. J. (2012). What is parental care. *The Evolution of Parental Care*, 1–14.

Smith, R. G., Kember, R. L., Mill, J., Fernandes, C., Schalkwyk, L. C., Buxbaum, J. D., & Reichenberg, A. (2009). Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: A mouse model. *PLoS ONE*, *4*(12), 4–8. https://doi.org/10.1371/journal.pone.0008456

Snowdon, C. T., & Pickhard, J. J. (1999). Family feuds: Severe aggression among cooperatively breeding cotton-top tamarins. *International Journal of Primatology*, *20*(5), 651–663. https://doi.org/10.1023/A:1020796517550

Snowdon, C. T., & Ziegler, T. E. (2007). Growing up cooperatively: family processes and infant care in marmosets and tamarins. *Journal of Developmental Processes*, *2*(1), 40–66.

Sparkman, A. M., Blois, M., Adams, J., Waits, L., Miller, D. A. W., & Murray, D. L. (n.d.). Evidence for sex-specific reproductive senescence in monogamous cooperatively breeding red wolves. https://doi.org/10.1007/s00265-016-2241-9

Stead, S., Mucha, S., & Bădescu, I. (2019). Alloparental Care. *Encyclopedia of Animal Cognition and Behavior*, (Wisenden 1999), 1–7. https://doi.org/10.1007/978-3-319-47829-6_1400-1

Stiver, K. A., & Alonzo, S. H. (2011). Alloparental care increases mating success. *Behavioral Ecology*, *22*(1), 206–211. https://doi.org/10.1093/beheco/arq186

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Hormones and Behavior*, *77*, 204–210. https://doi.org/10.1016/j.yhbeh.2015.07.005

Stone, A. I., Mathieu, D., Griffin, L., & Bales, K. L. (2010). Alloparenting experience affects future parental behavior and reproductive success in prairie voles (Microtus ochrogaster). *Behavioural Processes*, *83*(1), 8–15. https://doi.org/10.1016/j.beproc.2009.08.008

Storey, A. E., & Walsh, C. J. (2013). Biological basis of mammalian paternal behavior. *Handbook of Father Involvement: Multidisciplinary Perspectives*, *2*, 3–22.

Swartz, K. B., & Rosenblum, L. A. (1981). The social context of parental behavior. In *Parental care in mammals* (pp. 417–454). Springer.

Tidière, M., Thevenot, X., Deligiannopoulou, A., Douay, G., Whipple, M., Siberchicot, A., … Lemaître, J. F. (2018). Maternal reproductive senescence shapes the fitness consequences of the parental age difference in ruffed lemurs. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1886). https://doi.org/10.1098/rspb.2018.1479

Trainor, B. C., & Marler, C. A. (2001). Testosterone, paternal behavior, and aggression in the monogamous California mouse (Peromyscus californicus). *Hormones and Behavior*, *40*(1), 32–42. https://doi.org/10.1006/hbeh.2001.1652

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In R. L. Trivers (Ed.), *Sexual Selection and the Descent of Man* (pp. 136–179). Routledge. https://doi.org/10.4324/9781315129266-7

Trivers, R. L. (1974). Parent-Offspring Conflict. *American Zoologist*, *14*(1), 249–264. https://doi.org/10.1093/icb/14.1.249

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, *45*(3), 1–67. https://doi.org/10.18637/jss.v045.i03

Vleck, C. M., Vleck, D., & Palacios, M. G. (2011). Evolutionary ecology of senescence: A case study using tree Swallows, Tachycineta bicolor. *Journal of Ornithology*. https://doi.org/10.1007/s10336-010-0629-2

Wilcoxen, T. E., Boughton, R. K., & Schoech, S. J. (2010). Older can be better: Physiological costs of paternal investment in the Florida scrub-jay. *Behavioral Ecology and Sociobiology*, *64*(10), 1527–1535. https://doi.org/10.1007/s00265-010-0966-4

Wittenberger, J. F., & Tilson, R. L. (1980). The evolution of monogamy: hypotheses and evidence. *Annual Review of Ecology and Systematics*, *11*(1), 197–232.

Woodroffe, R., & Vincent, A. (1994). Mother' s little helpers: patterns of male care in mammals, (8), 294–297.

Wu, R., Song, Z., Tai, F., An, X., Yu, P., & Li, Y. (2013). The effect of alloparental experience and care on anxiety-like, social and parental behaviour in adult mandarin voles. *Animal Behaviour*, *85*(1), 61–69. https://doi.org/10.1016/j.anbehav.2012.10.007

Wu, R., Song, Z., Tai, F., Wang, L., Kong, L., & Wang, J. (2013). Post-weaning living with parents during juvenile period alters locomotor activity, social and parental behaviors in mandarin voles. *Behavioural Processes*, *98*, 78–84. https://doi.org/10.1016/j.beproc.2013.05.008

Xie, K., Ryan, D. P., Pearson, B. L., Henzel, K. S., Neff, F., Vidal, R. O., … Ehninger, D. (2018). Epigenetic alterations in longevity regulators, reduced life span, and exacerbated aging-related pathology in old father offspring mice. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), E2348–E2357. https://doi.org/10.1073/pnas.1707337115

Zhao, M., Garland, T., Chappell, M. A., Andrew, J. R., Saltzman, W., Garland Jr, T., … Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiology and Behavior*, *177*(April), 57–67. https://doi.org/10.1016/j.physbeh.2017.04.010

Ziegler, T. E. (2000). Hormones associated with non-maternal infant care: A review of mammalian and avian studies. *Folia Primatologica*, *71*(1–2), 6–21. https://doi.org/10.1159/000021726

Zöttl, M., Fischer, S., & Taborsky, M. (2013). Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour*, *85*(6), 1471–1478. https://doi.org/10.1016/j.anbehav.2013.03.045

Chapter 1

Sex Differences in Offspring Discrimination in the Biparental California Mouse (*Peromyscus californicus*)

Abstract

Costs of parental care can include increased physical, physiological, and behavioral demands and reduced prospects for future reproduction. Therefore, animals may benefit from recognizing and discriminating their own offspring during the period of infant care to ensure that they provide care only to their own young. Differences in offspring discrimination between mothers and fathers may arise due to differences in the amount of parental care they provide. In mammals, mothers bear the costs of gestation and lactation, while fathers do not; therefore, we might expect that in mammal species in which both parents provide parental care, mothers will show greater levels of offspring discrimination than fathers. In the present study, we examined possible sex differences in offspring discrimination in the biparental, monogamous California mouse (Peromyscus californicus) across the lactational period. On postpartum days (PPD) 3, 7, 16, and 28, parents were housed individually in a test cage and presented simultaneously with two wire mesh balls, one containing their own pup and the other containing an unrelated pup. On individual test days, parents behaved similarly toward the two balls. Fathers, but not mothers, showed significant changes in latencies to approach and interact with the non-kin ball across test days, but no clear pattern was observed. Fathers also approached the non-kin ball more frequently than mothers when pups reached weaning age (PPD 28).

Thus, fathers may show increased interest in unrelated young as their offspring become independent.

1. Introduction

Individual recognition of conspecifics can provide a basis for ongoing social interactions, such as altruistic behavior, pairbonding, social hierarchy, and territorial defense (Mateo 2004; Ophir 2017; Carlson et al. 2020). Often, this recognition involves differentiating between related and unrelated conspecifics, leading to kin discrimination. Although recognition and discrimination are often closely associated with one another, they differ in that recognition refers to cognitive and neural processes of categorizing other individuals, while discrimination refers to differential behavior towards individuals (Tang-Martinez 2001; Holmes 2004). Discriminatory behavior can be evidence of recognition, but the absence of differential behaviors toward others does not necessarily indicate a lack of recognition ability (Tang-Martinez 2001).

For many species, parental care is an especially critical form of social interaction that may enhance offspring and, consequently, own fitness. Parental care is costly, as it can increase physical, physiological, and behavioral demands on an individual and reduce the prospects for future reproduction (Jönsson et al. 1998). Therefore, animals may benefit from recognizing and discriminating their own offspring during the period of post-natal care to ensure that only related infants receive care. Additionally, offspring recognition may prevent otherwise infanticidal adults from killing their own offspring (Huck et al. 1982).

Given the costs of parental care, we might expect that the sex that provides greater levels of care within a species will demonstrate greater levels of offspring discrimination. This has been observed in razorbills (*Alca torda*), in which fathers, the sole caregivers, discriminate between the calls of their own and unrelated chicks while mothers do not (Insley et al. 2003). Similarly, in species in which one sex experiences a greater risk of providing care to unrelated infants, that sex should be more discriminating in parental behavior. Ringler et al. (2016) observed that male brilliant-thighed poison frogs (Allobates femoralis) transport both related and unrelated tadpoles between pools of water, while females transport only their own tadpoles. The authors attributed this sex difference to the fact that males defend territories containing pools of water, and therefore, any tadpoles in a male's territory are likely his own offspring, while females risk transporting unrelated tadpoles from any given pool (Ringler et al. 2016).
Mammals provide a valuable taxon in which to investigate sex differences in offspring discrimination because they have an implicit, significant sex bias in parental costs: females must gestate and lactate while males do not. Thus, we expect that mammalian mothers experience greater costs than fathers of misidentifying young as their own, leading to sex differences in offspring-discrimination abilities (Holmes 1990). At a proximate level, social recognition, in at least sheep and rodents, is influenced by the neurohormone oxytocin, which rises dramatically in mothers during parturition and lactation (Bielsky and Young 2004). Fathers, too, can undergo changes in oxytocin signaling; however, these changes appear to be less pronounced and less consistent across species than those in mothers, and their functional significance is generally not known

(Saltzman and Ziegler 2014; Horrell et al. 2018). Therefore, the neuroendocrine changes that occur at the onset of motherhood in mammals may lead to differences in offspring-discrimination abilities between mothers and fathers.

In mammals, maternal discrimination of offspring has been observed in several taxa, including ungulates, primates, rodents, canids, pinnipeds, and bats (Halpin 1991; Keverne and Kendrick 1992; Solomon 1993; Hepper 1994; Maestripieri and Call 1996; Lévy et al. 2004; Kitchen and Knowlton 2006; Breed 2014; Padilla De La Torre et al. 2016; Carlson et al. 2020). In contrast, offspring discrimination by mammalian fathers has been examined in rodents [e.g. house mice (*Mus musculus*), Ostermeyer & Elwood 1983; Mak & Weiss 2010; striped mice (*Rhabdomys pumilio*), Pillay 2002; Brandt's voles (*Lasiopodomys brandtii*), Li & Zhang 2010; prairie voles (*Microtus ochrogaster*), Phillips & Tang-Martinez 1998; mandarin voles (*M. mandarinus*), Wang & Tai 2012], primates (reviewed in Widdig 2007), and carnivores [spotted hyenas (*Crocuta crocuta*, Van Horn, Wahaj, & Holekamp 2004)]. This relative dearth of research on offspring discrimination by fathers is likely due to the fact that mothers in all mammalian species provide care for their offspring (i.e., maternal care), while only approximately 5-10% of species display systematic care by fathers (i.e., paternal care) (Kleiman and Malcolm 1981).

Several studies in rodents have directly compared offspring discrimination in mammalian mothers and fathers. A study in the biparental Brandt's vole demonstrated that parents of both sexes were less aggressive towards familiar pups than unfamiliar pups, but only fathers were significantly more amicable to familiar pups, regardless of genetic relatedness (Li and Zhang 2010). A study in the facultatively biparental house mouse found that both mothers and fathers sniffed and licked unrelated pups more than their own pups; no sex differences were observed (Ostermeyer and Elwood 1983). Similarly, Phillips and Tang-Martinez (1998) observed that biparental prairie vole dyads consisting of one parent and one offspring displayed more investigatory and less agonistic behavior than dyads consisting of an adult with unrelated young, but no effects of adult sex were observed (Phillips and Tang-Martinez 1998).

Selection is predicted to favor the evolution of offspring discrimination by parents in polygamous and/or group-living species that have motile infants more than in monogamous and/or solitary species with altricial young (Gubernick 1981). Indeed, most observations of father-offspring recognition and discrimination in mammals have been in polygamous and/or highly social species in which fathers do not routinely provide care for their offspring (Pillay 2002; Widdig 2007; Li and Zhang 2010; Mak and Weiss 2010; Wang and Tai 2012). Furthermore, unlike mother-offspring discrimination, father-offspring discrimination in mammals has often been examined in the context of infanticide or inbreeding rather than paternal care (Huck et al. 1982; Elwood 1994; Pillay 2002, but see Mak and Weiss 2010). Given that paternal care in mammals is associated strongly, although not exclusively, with monogamy (Clutton-Brock 1991; Møller 2003), father-offspring discrimination might not be predicted to occur in a biparental mammal. Nonetheless, offspring discrimination by fathers has been observed in the monogamous and biparental prairie vole and in monogamous birds (Barg and Mumme 1994; Benedict 2007) and reptiles (Main and Bull 1996). A complete understanding of the evolution and ecology of offspring discrimination requires the assessment of species both that are and are not predicted to discriminate between their own offspring and those of conspecifics (Carlson et al. 2020).

In this study, we examined parent-offspring discrimination across the parental care-giving period in a biparental, monogamous rodent, the California mouse (Peromyscus californicus). Females give birth to litters of 1-4 pups after a gestation of approximately 31-33 days, followed by postpartum estrus and a lactational period lasting approximately 28 days (Gubernick 1988; unpublished data). Paternal care consists of the same behaviors as maternal care (e.g., grooming, licking, retrieving, and warming pups) with the exception of nursing (Gubernick and Alberts 1987). However, mothers and fathers do not necessarily provide the same amounts of care throughout the lactational period: females devote more time to pup care than fathers during the first five days postpartum, with males gradually increasing the amount of care they provide during this period (Rosenfeld et al. 2013). Thereafter, the parents spend similar amounts of time in the nest and in physical contact with their pups, but fathers groom pups more than mothers, and mothers perform higher levels of anogenital licking than fathers starting 17 days postpartum (Gubernick and Alberts 1987).

Although offspring discrimination in this species has not been examined directly, Gubernick et al. (1994) observed that some fathers housed with their mate and offspring exhibit infanticidal behavior towards unfamiliar pups. On the other hand, in cross-fostering experiments between California mice and a congeneric species, P. leucopus, Bester-Meredith and Marler (2001) found no differences in parental behavior between animals caring for their own or foster pups. Pups in these studies were wiped clean of all scents and then coated with bedding from the foster parents before being introduced, to ensure their acceptance by foster parents (Bester-Meredith and Marler 2001, 2003, 2007, 2012); however, the extent to which this procedure contributed to acceptance of foster pups is not stated. Thus, it remains unclear whether or not offspring discrimination occurs in this species.

We tested the hypothesis that offspring discrimination differs between mothers and fathers and changes across the postpartum period in the California mouse. We predicted that both parents would discriminate between own and unrelated pups, that mothers would display higher levels of discrimination than fathers, and that parents would show greater levels of offspring discrimination once their pups become motile at approximately 16 days of age (Gubernick and Alberts 1987).

2. Methods

2.1. Animals -

All animals in this study were descendants of California mice purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA) and were bred at the University of California, Riverside (UCR). Families (mated pair and offspring) were housed in polycarbonate cages (44 x 24 x 20 cm) with aspen shavings as bedding and cotton for nesting material. Animals were housed under a 14:10 light:dark cycle (lights on at 0500 h, lights off at 1900 h) at approximately 22°C. Food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA) and water were provided ad lib. Cages were checked twice daily for animal health and births, and bedding and water were changed weekly.

All procedures were approved by the UCR Institutional Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals. UCR is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2. Experimental Design -

Twelve breeding pairs (N=24 parents) were used. All tests were conducted when pairs were housed with their third litter. Each parent was tested for pup discrimination on postpartum days (PPD) 3, 7, 16, and 28 between 1300 and 1500 h. Pairmates were tested on the same day in randomized order.

2.3. Discrimination Test -

Mice were tested in a sound-reduced environmental chamber lit by a ceiling lamp. Tests were conducted in a clean polycarbonate cage, identical to the home cage, containing aspen shaving and cotton. Two stainless steel, wire mesh tea balls were suspended in opposite, diagonal corners of the cage from a transparent plastic cage lid (Fig. 1). Pups were placed in tea balls to ensure their safety in case a focal animal behaved aggressively as well as to prevent older pups from moving towards or away from the focal animal. For tests on PPD 3 and 7 we used balls that were 5.1 cm in diameter, and for tests on PPD 16 and 28 we used balls that were 7.6 cm in diameter. Clean cotton was added to each tea ball to provide warmth for the pups and therefore to reduce pup vocalizations, as low temperatures increase vocalizations in rodent pups (Blumberg and Alberts 1990; Szentgyörgyi et al. 2008).

At the beginning of each test, one parent was placed in the test cage for a 15-min habituation period. We then briefly removed the parent and placed a pup from the parent's litter ("kin") in one tea ball and an unrelated pup ("non-kin") in the other ball. The two pups were born within two days of each other and were similar in size, although we did not weigh pups before each test. The locations of the two pups were randomized across tests. The parent was immediately returned to the test cage and allowed to explore the tea balls for 10 min before being reunited with its mate and offspring in its home cage. After the test, all animals were returned to their home cages. One hour later, the second parent was tested identically in a clean test cage with fresh cotton and bedding. When possible, different stimulus pups were used with the two parents on a given test day. We were unable to control whether the same non-kin stimulus pups were used across consecutive tests days. All trials were video-recorded using a GoPro Hero-6 camera (GoPro; San Mateo, California, USA) suspended above the test cage.

2.4. Video Analysis -

Videos were analyzed using TopScan tracking software (CleverSys, Reston, VA, USA) to track the movement of the parent throughout each trial. TopScan generated two concentric circular regions centered on each tea ball: a smaller arena with a radius of approximately one head length (25.4 mm) and a larger arena with a radius of approximately one body length (101.6 mm); Fig. 1) (Merritt 1978). Time spent in the smaller arena was used an index of physical contact with or olfactory investigation of the stimulus (ball + pup), and time spent in the larger arena, but outside the smaller one, was used as an index of interest in the stimulus. TopScan logged each occasion on which the nose of the mouse entered each arena. The following behaviors were scored for each stimulus: latency to approach (i.e., to enter the larger arena), latency to interact (i.e., to enter the smaller arena), duration of time spent in the larger arena, and duration of time spent in the smaller arena. We also recorded which stimulus the adult subject interacted with first. All videos were scored by a single observer who was blind to the identity of the test subjects and the location of the kin and non-kin pups.

2.5. Data Analysis -

The data were not normally distributed and variances were not homogeneous; therefore, we used nonparametric statistical tests. Data were analyzed using SPSS version 24.0 (IBM 2016). For each time point we used exact Wilcoxon signed-rank and McNemar's tests to compare behaviors between mothers and fathers and to compare behaviors directed towards the non-kin and kin balls. We used Friedman and Cochran's Q tests to evaluate longitudinal changes in each behavior, both for each sex individually and for the sexes combined. Following significant tests, we performed post-hoc analyses using the method recommended by Sokal and Rohlf (1995) for Friedman tests and McNemar's test for Cochran's Q, respectively. We set alpha at 0.05 (two-tailed).

3. Results

One mother died from dehydration on PPD 25 due to a faulty water bottle that was introduced five days prior to her death; thus, data from this animal and her mate were analyzed only for PPD 3, 7, and 16. Another female remained stationary during the entire test session on each of the four test days. Therefore, she and her mate were excluded from analyses of sex differences, and the female was also excluded from all other analyses. Finally, data from four tests (two from PPD7, one from PPD16, and one from PPD28) could not be used as a result of technical problems during testing. Final sample sizes are shown in Table 1.

When parents were placed in the test cage following introduction of the two stimuli, they typically approached and investigated one of the balls within 2 minutes. For the remainder of the test, most animals moved frequently between the two balls, spending most of their time sniffing the stimuli. Several animals handled the balls and appeared to try to remove the pups. However, this behavior did not occur frequently enough to be scored.

To determine whether the order of testing (i.e. whether the mother or father of a pair was tested first) had an effect on the parents' behavior during the test, we performed exact Wilcoxon and McNemar's tests comparing data from parents that were tested first and those tested second for each test day. On PPD 16, animals that were tested first had shorter latencies to approach the non-kin ball than animals that were tested second ($T+ = 40$, $p = 0.039$). No other significant differences were found.

3.1. Responses to Kin vs. Non-Kin -

When analyzed separately, neither mothers nor fathers showed differential behavioral responses to the kin and non-kin balls on any test day (Table 1). When data from mothers and fathers were pooled, however, the latency to approach the non-kin ball was significantly shorter than latency to approach the kin ball on PPD 16 (T+ = 162, p = 0.033, Wilcoxon test). Similarly, parents were more likely to first approach the non-kin ball rather than the kin ball on PPD 16 ($\chi 2 = 5.000$, p = 0.025, McNemar's test). No other significant differences between responses to the two balls were observed using the combined data from both parents (Table 1).

3.2. Sex Differences -

No significant sex differences were observed in any behaviors on PPD 3, 7, or 16 (Table 1). On PPD 28, fathers entered the larger arena around the non-kin ball sooner (T+ = 34, p = 0.023, Wilcoxon test) and more frequently than mothers (T+ = 34, p = 0.023, Wilcoxon test). No other significant differences between mothers and fathers were observed on PPD 28 (Table 1).

3.3. Longitudinal Changes -

Fathers showed a significant change in latency to approach ($\chi 2 = 8.696$, p = 0.034) and interact with ($\chi 2 = 10.050$, p = 0.018) the non-kin ball. These latencies were lowest on PPD 7 and 16 compared to PPD 3 and 28; however, post-hoc analyses did not yield any significant pairwise comparisons for either variable. A significant longitudinal change was also found in which ball the fathers approached first ($Q = 10.111$, $p = 0.018$, Cochran’s Q): more fathers approached the unrelated pup first on PPD 7 than on PPD 3 ($\chi2 = 0.750$, $p = 0.028$, McNemar’s test). No changes across test days were observed in mothers.

When we analyzed mothers and fathers together, we found a significant change in latency to approach ($\chi2 = 14.493$ $p = 0.002$, Friedman test) and latency to interact with ($\chi2 = 12.200$, $p = 0.007$) the ball containing the non-kin pup. Animals approached and interacted with the non-kin ball earliest on PPD 16 compared to the other test days, but post-hoc analyses for the latency to approach did not yield any significant pairwise comparisons. Examining the latency to interact, we observed that on PPD 28, animals took significantly longer to interact with the ball containing the non-kin pup than they did on PPD 16 ($U = 193$, $p < 0.005$; Fig. 2) but not on PPD 3 or 7.

Significant longitudinal changes were also observed in which stimulus the parents interacted with first ($Q = 9.000$, $p = 0.029$, Cochran’s Q). Pairwise comparisons revealed that more animals interacted with the non-kin ball first on PPD 16 than on PPD 3 ($\chi2 = -0.500$, $p = 0.044$, NcNemar’s test, Fig. 3). Significant changes over time were not observed for any other measure with the pooled data set from mothers and fathers.

4. Discussion

In this study we tested the hypothesis that California mouse parents discriminate their own offspring from unrelated young, and that mothers display greater levels of offspring discrimination than fathers. Overall, we observed no sex differences in behavior towards either pup-containing tea ball on individual test days. Fathers, but not mothers, displayed significant longitudinal changes in behaviors directed towards the non-kin ball, but these changes did not follow a consistent pattern.

4.1. Offspring Discrimination in California Mice -

On postpartum day 16, parents approached the ball containing an unfamiliar pup sooner than the ball containing their own pup and, correspondingly, were more likely to approach the non-kin ball first. However, no other significant differences were found in behaviors directed towards the two balls, suggesting that offspring discrimination is not pronounced in California mice. This result might be explained by the natural history of our study species. California mice are highly territorial and monogamous, although extrapair copulation may occasionally occur (Gubernick and Nordby 1993). The differences in behavior toward kin and non-kin on day 16 coincide with the increase in pup motility at that age (Gubernick and Alberts 1987). However, under natural conditions, pups do not disperse from the natal home range until approximately 35 days of age (Ribble 1992). Therefore, our results are consistent with Gubernick's (1981) hypothesis that offspring discrimination is more likely to evolve in highly social, polygamous species with motile young than in monogamous, territorial species with immotile young.

We did not assess pup recognition per se in this study, which leaves open the possibility for offspring recognition without display of offspring discrimination. Our test subjects might not have been motivated to behave differently towards the two pups because we used a relatively small experimental setup; the two balls were close to each other, allowing animals to move easily between them. Additionally, we did not allow the animals to physically interact with the pups. Permitting the parents to have physical contact with the stimulus pups and measuring more nuanced behavioral variables might reveal subtle differences in how adults respond to their own vs. unrelated pups. Moreover, for logistical reasons, we were not able to account for the sexes of either kin or non-kin pups. Therefore, we cannot determine whether or to what extent the parents' behavior was affected by the pups' sexes. Finally, we note that our sample sizes were relatively small, especially by PPD 28, which reduced our statistical power to detect offspring discrimination.

4.2. Longitudinal Changes in Parents' Responses to Pups -

We found significant longitudinal changes in the latency to approach and interact with the non-kin ball and in which stimulus the animals interacted with first: when mothers and fathers were analyzed together, they had shorter latencies to approach and interact with the non-kin ball on PPD 16 compared to the other three test days. Additionally, more parents interacted with the non-kin ball before the kin ball on PPD 16 than on PPD 3. The same three longitudinal patterns were observed in fathers alone, but not in mothers alone.

These longitudinal changes might suggest that California mouse fathers become more interested in investigating non-kin pups over time. Previous studies in prairie voles and cooperatively breeding Belding's ground squirrels (Spermophilus beldingi) found that mothers discriminate between own and unrelated weanlings, but not between own and unrelated younger pups (Holmes and Sherman 1982; Hayes et al. 2004). However, we observed that data from PPD 16, but not PPD 28 (i.e., age of weaning), differ from those on other days. It is unclear why the results from PPD 28 did not differ from those of earlier test days.

It is possible that increasing discrimination between own and unrelated offspring across the developmental period might not be adaptive per se but might simply reflect an increase in parents' ability to discriminate between offspring, if, for example, individual differences in offspring vocalizations or odors emerge with age (Beecher et al. 1981; Jovanovic et al. 2000; Mateo 2006). Prior to weaning, California mouse pups spend virtually all their time attached to the mother's nipples, at least in a lab setting, which might eliminate the parents' ability to discriminate among individual pups. We also cannot discount the possibility that increased interest in the non-kin pup over time was related to the novelty of the pup, rather than to social or parental motivation. Rodents are attracted to novelty (Mitchell 1976), which may explain why all significant results in this study involved behaviors directed towards the non-kin ball.

Another possible explanation for the longitudinal changes we observed is related to territorial defense and/or parental aggression in California mice. In this species, males and females defend territory from intruders, and both pairmates display similar levels of aggression, regardless of intruder sex (Ribble and Salvioni 1990; Rieger and Marler 2018; Rieger et al. 2019). Possibly, parents in this study approached unrelated pups sooner on later test days because the older pups were perceived as potentially infanticidal intruders.

An important caveat is that parents in our study were often tested with pups from the same unrelated litter across test days due to constraints on animal availability. Therefore, parents likely encountered the same non-kin pup during multiple 10-minute tests, which would have reduced the novelty of these pups. Unfortunately, we were not able to statistically compare responses to non-kin pups on the first versus subsequent encounters because we were unable to identify individual pups over the course of the study. Moreover, because almost all parents were exposed to pups from the same non-kin litter in multiple tests, sample sizes for these analyses would have been insufficient.

4.3. Sex Differences in Parents' Responses to Pups -

In biparental species, sex differences in the types and amount of parental care provided and in the neuroendocrine processes that mediate social recognition might lead to differences in offspring discrimination between mothers and fathers. However, we found little evidence for sex differences in offspring discrimination on individual test days. Behavior differed between mothers and fathers only on PPD 28, when fathers approached the ball containing the non-kin pup sooner and more frequently than did mothers. Thus, fathers appeared to be more attracted to non-kin pups than mothers when their offspring reached the age of weaning.

As described above, fathers, but not mothers, showed a decrease in latency to approach the non-kin ball over time, as well as an increase in likelihood of approaching the non-kin ball first. Male rats and house mice display more social investigation (Thor 1980; Karlsson et al. 2015), as well as more pronounced habituation to novelty (i.e. greater decline in investigatory behavior over multiple exposures), compared to females (Frick and Gresack 2003; Reeb and Tang 2005). On the other hand, Agarwal et al. (2020) observed that female California mice have greater object recognition memory than males and that this sex difference is most apparent when the two stimulus objects are similar (Agarwal et al. 2020). Thus, the differences we observed between mothers' and fathers' responses to kin and non-kin over time might reflect sex differences in recognition of and attraction to stimuli.

In summary, adult California mice showed relatively few differences in their behavioral responses to their own and unfamiliar pups. In the second half of the lactational period, however, fathers showed greater interest in or attraction to unrelated pups than to their own offspring, whereas no such pattern was seen in mothers. These findings contrast with our prediction that offspring discrimination would be more pronounced in mothers than in fathers. However, our results are consistent with our expectation that parents display more discrimination during the latter part of the lactational period than earlier. The mechanisms underlying these sex- and time-dependent changes in offspring discrimination, possibly including experiential, neuroendocrine, and sensory changes, await further study.

References

Agarwal P, Palin N, Walker SL, Glasper ER (2020) Sex-dependent effects of paternal deprivation and chronic variable stress on novel object recognition in adult California mice (Peromyscus californicus). Horm Behav 117:104610. https://doi.org/10.1016/j.yhbeh.2019.104610

Barg JJ, Mumme RL (1994) Parental recognition of juvenile begging calls in the Florida scrub jay. Auk 111:459–464. https://doi.org/10.2307/4088610

Beecher MD, Beecher IM, Hahn S (1981) Parent-offspring recognition in bank swallows (Riparia riparia): II. Development and acoustic basis. Anim Behav 29:95–101. https://doi.org/10.1016/S0003-3472(81)80156-X

Benedict L (2007) Offspring discrimination without recognition: California towhee responses to chick distress calls. Condor 109:79. https://doi.org/10.1650/0010-5422(2007)109[79:odwrct]2.0.co;2

Bielsky IF, Young LJ (2004) Oxytocin, vasopressin, and social recognition in mammals. Peptides 25:1565–1574. https://doi.org/10.1016/j.peptides.2004.05.019

Blumberg MS, Alberts JR (1990) Ultrasonic vocalizations by rat pups in the cold: an acoustic by-product of laryngeal braking? Behav Neurosci 104:808–817. https://doi.org/10.1037/0735-7044.104.5.808

Breed MD (2014) Kin and nestmate recognition: The influence of W. D. Hamilton on 50 years of research. Anim Behav 92:271–279. https://doi.org/10.1016/j.anbehav.2014.02.030

Carlson N V., Kelly EMK, Couzin I (2020) Individual vocal recognition across taxa: a review of the literature and a look into the future. Philos Trans R Soc Lond B Biol Sci 375:20190479. https://doi.org/10.1098/rstb.2019.0479

Clutton-Brock TH (1991) The Evolution of Parental Care. Princeton University Press, Princeton, New Jersey, USA

de Jong TR, Chauke M, Harris BN, Saltzman W (2009) From here to paternity: Neural correlates of the onset of paternal behavior in California mice (Peromyscus californicus). Horm Behav 56:220–231. https://doi.org/10.1016/j.yhbeh.2009.05.001

Elwood RW (1994) Temporal-based kinship recognition: A switch in time saves mine. Behav Processes 33:15–24. https://doi.org/10.1016/0376-6357(94)90057-4

Frick KM, Gresack JE (2003) Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice. Behav Neurosci 117:1283–1291. https://doi.org/10.1037/0735-7044.117.6.1283

Gubernick DJ (1981) Parent and Infant Attachment in Mammals. In: Gubernick DJ, Klopfer PH (eds) Parental Care in Mammals. Springer US, Boston, MA, pp 243–305

Gubernick DJ (1988) Reproduction in the California Mouse, Peromyscus californicus. J Mammal 69:857–860. https://doi.org/10.2307/1381649

Gubernick DJ, Alberts JR (1987) The biparental care system of the California mouse, Peromyscus californicus.. J Comp Psychol 101:169–177. https://doi.org/10.1037/0735-7036.101.2.169

Gubernick DJ, Nordby JC (1993) Mechanisms of sexual fidelity in the monogamous California mouse, Peromyscus californicus. Behav Ecol Sociobiol 32:211–219. https://doi.org/10.1007/BF00173779

Halpin ZT (1991) Kin recognition cues of vertebrates. In: Hepper PG (ed) Kin Recognition. Cambridge University Press, pp 220–258

Hayes LD, O'Bryant E, Christiansen AM, Solomon NG (2004) Temporal changes in mother–offspring discrimination in the prairie vole (Microtus ochrogaster). Ethol Ecol Evol 16:145–156–145–156. https://doi.org/10.1080/08927014.2004.9522643

Hepper PG (1994) Long-term retention of kinship recognition established during infancy in the domestic dog. Behav Processes 33:3–14. https://doi.org/10.1016/0376-6357(94)90056-6

Holmes WG (2004) The early history of Hamiltonian-based research on kin recognition. Ann Zool Fennici 41:691–711

Holmes WG, Sherman PW (1982) The ontogeney of kin recognition in two species of ground squirrels. Am Zool 22:491–517

Horrell ND, Hickmott PW, Saltzman W (2018) Neural regulation of paternal behavior in mammals: sensory, neuroendocrine, and experiential influences on the paternal brain. In: Coolen LM, Grattan DR (eds) Neuroendocrine Regulation of Behavior. Springer International Publishing, Basel, Switzerland, pp 111–160

Huck UWi, Soltis RL, Coopersmith CB (1982) Infanticide in male laboratory mice: Effects of social status, prior sexual experience, and basis for discrimination between related and unrelated young. Anim Behav 30:1158–1165. https://doi.org/10.1016/S0003-3472(82)80206-6

Insley SJ, Paredes R, Jones IL (2003) Sex differences in razorbill Alca torda parent--offspring vocal recognition. J Exp Biol 206:25–31. https://doi.org/10.1242/jeb.00072

Jönsson KI, Tuomi J, Järemo J, et al (1998) Pre- and postbreeding costs of parental investment. Oikos 83:424. https://doi.org/10.2307/3546670

Jovanovic T, Megna NL, Maestripieri D (2000) Early maternal recognition of offspring vocalizations in rhesus macaques (Macaca mulatta). Primates 41:421–428. https://doi.org/10.1007/BF02557653

Karlsson SA, Haziri K, Hansson E, et al (2015) Effects of sex and gonadectomy on social investigation and social recognition in mice. BMC Neurosci 16:1–10. https://doi.org/10.1186/s12868-015-0221-z

Keverne EB, Kendrick KM (1992) Oxytocin facilitation of maternal behavior in sheep. Ann N Y Acad Sci 652:83–101. https://doi.org/10.1111/j.1749-6632.1992.tb34348.x

Kitchen AM, Knowlton FF (2006) Cross-fostering in coyotes: Evaluation of a potential conservation and research tool for canids. Biol Conserv 129:221–225. https://doi.org/10.1016/j.biocon.2005.10.036

Kleiman DG, Malcolm JR (1981) The evolution of male parental investment in mammals. In: Parental Care in Mammals. Springer US, Boston, MA, pp 347–387

Lee AW, Brown RE (2002) Medial preoptic lesions disrupt parental behavior in both male and female California mice (Peromyscus californicus). Behav Neurosci 116:968–975. https://doi.org/10.1037/0735-7044.116.6.968

Lee AW, Brown RE (2007) Comparison of medial preoptic, amygdala, and nucleus accumbens lesions on parental behavior in California mice (Peromyscus californicus). Physiol Behav 92:617–628. https://doi.org/https://doi.org/10.1016/j.physbeh.2007.05.008

Lévy F, Keller M, Poindron P (2004) Olfactory regulation of maternal behavior in mammals. Horm Behav 46:284–302. https://doi.org/10.1016/j.yhbeh.2004.02.005

Li Q, Zhang L (2010) Parent-offspring recognition in Brandt's voles, Lasiopodomys brandti. Anim Behav 79:797–801. https://doi.org/10.1016/j.anbehav.2009.12.001

Maestripieri D, Call J (1996) Mother-infant communication in primates. Adv Study Behav 25:613–642. https://doi.org/10.1016/S0065-3454(08)60344-7

Main AR, Bull CM (1996) Mother-offspring recognition in two Australian lizards, Tiliqua rugosa and Egernia stokesii. Anim Behav 52:193–200. https://doi.org/10.1006/anbe.1996.0164

Mak GK, Weiss S (2010) Paternal recognition of adult offspring mediated by newly generated CNS neurons. Nat Neurosci 13:753–758. https://doi.org/10.1038/nn.2550

Mateo JM (2004) Recognition systems and biological organization: The perception component of social recognition. Ann. Zool. Fennici 41:729–745

Mateo JM (2006) Development of individually distinct recognition cues. Dev Psychobiol 48:508–519. https://doi.org/10.1002/dev.20156

Mitchell D (1976) Experiments on neophobia in wild and laboratory rats: A reevaluation. J Comp Physiol Psychol 90:190–197. https://doi.org/10.1037/h0077196

Møller AP (2003) The evolution of monogamy: mating relationships, parental care and sexual selection. In: Boesch C, Reichard UH (eds) Monogamy: mating strategies and partnerships in birds, humans and other mammals. Cambridge University Press Cambridge, UK, pp 29–41

Ophir AG (2017) Navigating monogamy: Nonapeptide sensitivity in a memory neural circuit may shape social behavior and mating decisions. Front Neurosci 11:397. https://doi.org/10.3389/fnins.2017.00397

Ostermeyer MC, Elwood RW (1983) Pup recognition in Mus musculus: Parental discrimination between their own and alien young. Dev Psychobiol 16:75–82. https://doi.org/10.1002/dev.420160202

Padilla De La Torre M, Briefer EF, Ochocki BM, et al (2016) Mother-offspring recognition via contact calls in cattle, Bos taurus. Anim Behav 114:147–154. https://doi.org/10.1016/j.anbehav.2016.02.004

Phillips ML, Tang-Martinez Z (1998) Parent-offspring discrimination in the prairie vole and the effects of odors and diet. Can J Zool 76:711–716. https://doi.org/10.1139/cjz-76-4-711

Pillay N (2002) Father-daughter recognition and inbreeding avoidance in the striped mouse, Rhabdomys pumilio. Mamm Biol 67:212–218. https://doi.org/10.1078/1616-5047-00032

Reeb BC, Tang AC (2005) Sex difference in temporal patterns of social interaction and its dependence upon neonatal novelty exposure. Behav Brain Res 158:359–365. https://doi.org/10.1016/j.bbr.2004.09.025

Ribble DO (1992) Dispersal in a monogamous rodent, Peromyscus californicus. Ecology 73:859–866. https://doi.org/10.2307/1940163

Ringler E, Pašukonis A, Ringler M, Huber L (2016) Sex-specific offspring discrimination reflects respective risks and costs of misdirected care in a poison frog. Anim Behav 114:173–179. https://doi.org/10.1016

Rosenfeld CS, Johnson SA, Ellersieck MR, Roberts RM (2013) Interactions between parents and parents and pups in the monogamous California mouse (Peromyscus californicus). PLoS One 8:e75725. https://doi.org/10.1371/journal.pone.0075725

Saltzman W, Ziegler TE (2014) Functional significance of hormonal changes in mammalian fathers. J Neuroendocrinol 26:685–696. https://doi.org/10.1111/jne.12176

Sokal RR, Rohlf FJ (1995) Nonparametric Methods in Lieu of Single-Classification ANOVA. In: Biometry, Third Edit. WH Freeman and Company, New York, NY, USA, pp 423–439

Solomon NG (1993) Preference for own versus conspecific pups by inbred and outbred rats. Behav Processes 30:317–322. https://doi.org/10.1016/0376-6357(93)90144-G

Szentgyörgyi H, Kapusta J, Marchlewska-Koj A (2008) Ultrasonic calls of bank vole pups isolated and exposed to cold or to nest odor. Physiol Behav 93:296–303. https://doi.org/10.1016/j.physbeh.2007.09.015

Tang-Martinez Z (2001) The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: A critical re-evaluation. Behav Processes 53:21–40. https://doi.org/10.1016/S0376-6357(00)00148-0

Thor DH (1980) Testosterone and persistence of social investigation in laboratory rats. J Comp Physiol Psychol 94:970–976. https://doi.org/10.1037/h0077831

Van Horn RC, Wahaj SA, Holekamp KE (2004) Role-reversed nepotistic interactions between sires and offspring in the spotted hyena. Ethology 110:1–14

Wang J, Tai F (2012) Effects of different periods of post-weaning separation on father-son interaction and recognition in mandarin voles. Ethol Ecol Evol 24:117–126. https://doi.org/10.1080/03949370.2011.597780

Widdig A (2007) Paternal kin discrimination: The evidence and likely mechanisms. Biol Rev 82:319–334. https://doi.org/10.1111/j.1469-185X.2007.00011.x

Table 1.1. Behaviors of mothers and fathers toward the kin and non-kin balls on postpartum days 3, 7, 16, and 28. All analyses were performed using exact Wilcoxon tests except analyses of first ball approached, which were performed using McNemar's test. Significant results (P<0.05) are in bold. Data shown are from all animals used in each analysis.

Behavior	**Group**	**PPD 3 (n = 11 mothers, 11 fathers)**			**PPD 7 (n = 10 mothers, 11 fathers)**		
		Kin	Non-Kin	Kin vs. Non-Kin (P)	Kin	Non-Kin	Kin vs. Non-Kin (P)
Duration in outer arena (s)	Mothers	68.60 (47.63, 114.00)	83.23 (58.63, 124.37)	0.557	73.47 (57.63, 156.42)	117.64 (75.65, 184.21)	0.322
	Fathers	85.43 (70.03, 116.00)	100.70 (54.07, 135.87)	0.970	63.40 (49.35, 80.40)	90.77 (47.46, 143.40)	0.365
	Mothers vs. Fathers (P)	0.365	0.520	--	0.426	0.652	--
Duration in inner arena (s)	Mothers	64.47 (26.20, 120.13)	99.8 (35.12, 126.47)	0.232	56.035 (23.77, 100.37)	69.30 (27.63, 102.97)	0.846
	Fathers	113.07 (52.93, 149.53)	75.00 (54.67, 123.87)	0.266	65.77 (30.08, 112.75)	109.165 (57.27, 142.43)	0.175
	Mothers vs. Fathers (P)	0.240	0.700	--	1.000	0.250	--
Entries into outer arena (no.)	Mothers	87 (58, 145)	118 (77, 180)	0.391	105 (58, 170)	128 (83, 150)	0.922
	Fathers	85 (77, 136)	94 (62, 130)	0.677	69 (52, 89)	121 (65, 136)	0.097
	Mothers vs. Fathers (P)	0.700	0.465	--	0.164	0.516	--
Entries into inner arena (no.)	Mothers	39 (24, 74)	82 (47, 93)	0.160	45 (15, 126)	60 (23, 81)	0.734
	Fathers	66 (33, 80)	73 (29, 89)	0.662	47 (27, 64)	54 (37, 107)	0.533
	Mothers vs. Fathers (P)	0.365	0.592	--	0.652	0.672	--

Table 1.1. cont.

[a] One female did not approach either ball during the entire duration of the test.

Latency to enter outer arena (approach) (s)	**Mothers**	**21.33 (0.00, 37.67)**	**5.90 (0.33, 51.47)**	**0.625**	**4.10 (2.54, 54.35)**	**10.87 (0.08, 26.94)**	**0.375**
	Fathers	5.23 (0.57, 28.23)	18.10 (6.83, 23.70)	0.151	24.57 (6.20, 60.26)	4.13 (1.20, 25.07)	0.175
	Mothers vs. Fathers (P)	0.966	0.898	--	0.570	0.715	--
Latency to enter inner arena (interact) (s)	Mothers	19.73 (1.03, 106.67)	6.33 (0.17, 112.47)	0.770	25.70 (4.99, 133.82)	24.87 (17.99, 75.87)	0.959
	Fathers	9.13 (1.93, 80.97)	21.33 (8.57, 57.33)	0.622	18.64 (4.24, 77.90)	15.12 (4.88, 54.50)	0.638
	Mothers vs. Fathers (P)	0.520	0.831	--	1.000	0.820	--
First ball approached	Mothers	5[a]	5[a]		7	3	
	Fathers	6	5		4	7	
	Mothers vs. Fathers (P)			1.000			0.125

Table 1.1 cont. [a] One female did not approach either ball during the entire duration of the test.

Behavior	**Group**	**PPD 16 (n = 10 mothers, 9 fathers)**			**PPD 28 (n = 8 mothers, 9 fathers)**		
		Kin	Non-Kin	Kin vs. Non-Kin (P)	Kin	Non-Kin	Kin vs. Non-Kin (P)
Duration in outer arena (s)	Mothers	108.52 (74.15, 187.14)	99.65 (65.59, 131.45)	0.922	134.09 (58.24, 221.14)	88.29 (68.35, 133.35)	0.313
	Fathers	111.43 (88.17, 177.42)	90.97 (75.97, 125.15)	0.432	103.93 (49.64, 137.25)	118.07 (90.55, 142.92)	0.557
	Mothers vs. Fathers (P)	0.910	0.734	--	0.461	0.313	--
Duration in inner arena (s)	Mothers	31.62 (20.45, 86.96)	43.09 (24.87, 121.83)	0.557	37.07 (12.69, 77.72)	39.47 (15.10, 145.25)	0.945
	Fathers	63.50 (48.27, 90.99)	59.47 (37.27, 78.25)	0.275	51.87 (31.80, 74.43)	49.60 (40.97, 126.59)	0.322
	Mothers vs. Fathers (P)	0.426	0.820	--	0.641	0.078	--
Entries into outer arena (no.)	Mothers	133 (66, 154)	120 (52, 173)	1.000	113 (71, 145)	51 (36, 74)	0.109
	Fathers	97 (67, 110)	90 (79, 146)	0.492	80 (54, 160)	96 (62, 154)	0.898
	Mothers vs. Fathers (P)	0.359	0.734	--	0.313	**0.023**	--
Entries into inner arena (no.)	Mothers	34 (29, 73)	47 (27, 111)	0.570	37 (28, 69)	25 (11, 54)	0.844
	Fathers	43 (29, 63)	53 (26, 73)	0.754	39 (35, 61)	40 (23, 94)	0.936
	Mothers vs. Fathers (P)	0.977	1.000	--	0.984	0.547	--

Table 1.1. cont.

Latency to enter outer arena (approach) (s)	**Mothers**	**10.73 (1.74, 48.15)**	**0.20 (0.02, 28.69)**	**0.432**	**26.27 (0.92, 60.15)**	**61.82 (18.22, 101.67)**	**0.383**
	Fathers	3.90 (0.32, 38.35)	1.60 (0.30, 11.59)	0.922	52.17 (0.48, 156.96)	13.69 (0.12, 26.00)	0.432
	Mothers vs. Fathers (P)	0.359	0.652	--	0.313	**0.023**	--
Latency to enter inner arena (interact) (s)	Mothers	41.92 (18.09, 88.34)	4.68 (1.15, 67.86)	0.160	35.97 (0.03, 79.42)	103.20 (52.48, 141.74)	0.195
	Fathers	58.50 (8.17, 105.70)	23.13 (2.83, 39.50)	0.105	83.37 (13.17, 173.90)	106.33 (30.12, 131.54)	0.922
	Mothers vs. Fathers (P)	0.425	0.945	--	0.195	0.844	--
First ball approached	Mothers	3	7		4	4	
	Fathers	2	7		6	3	
	Mothers vs. Fathers (P)			1.000			1.000

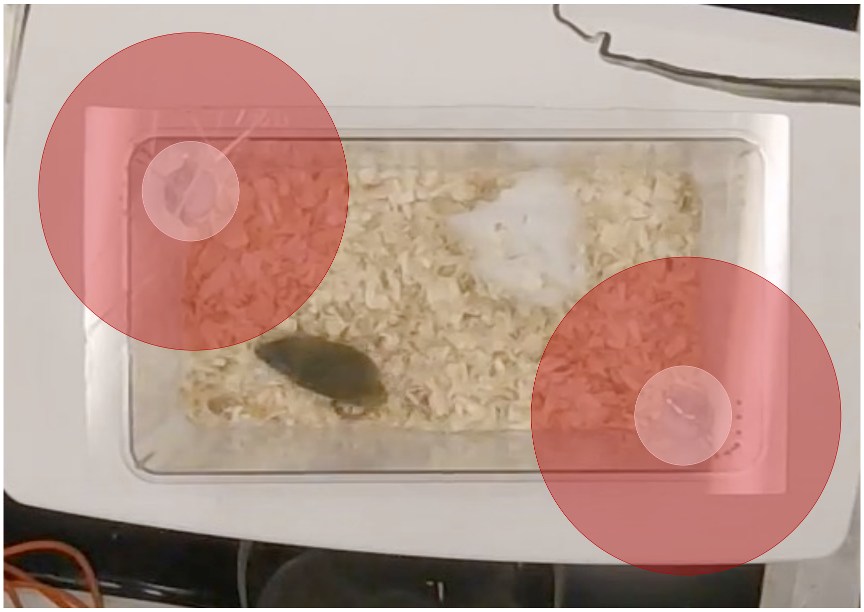

Figure 1.1. Photograph of test setup showing the test subject (parent) in the center of the cage and two wire mesh tea balls suspended from the cage lid in opposite corners, with each ball containing cotton and a kin (offspring) or non-kin pup. Computer-generated circles around each tea ball indicate the smaller and larger regions created in TopScan as proxies for interaction with and interest in the stimuli, respectively.

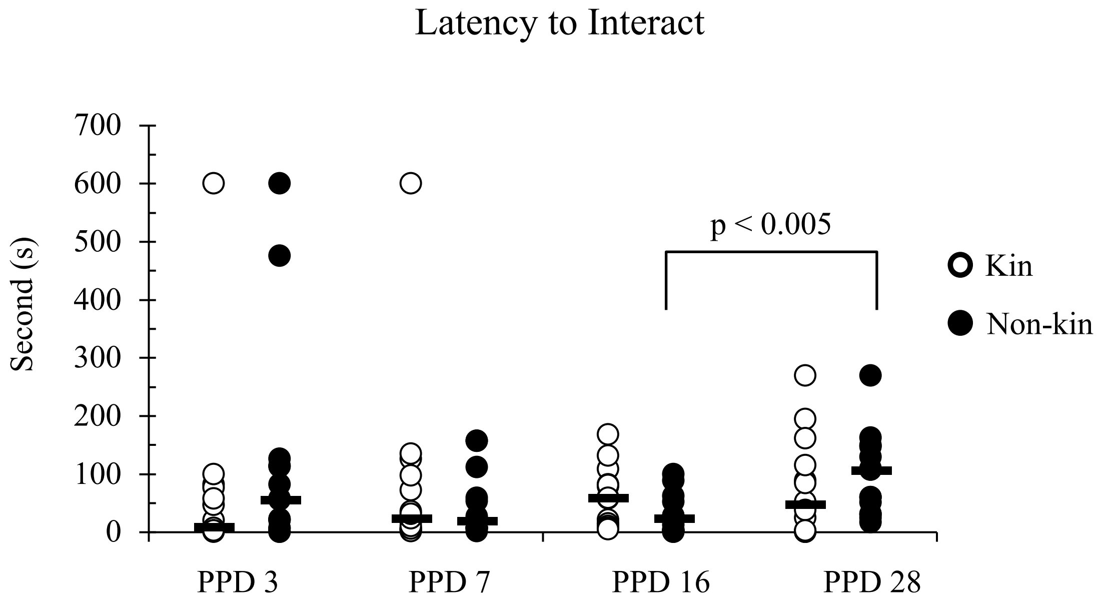

Figure 1.2. Latency to interact with the kin and non-kin ball by California mouse parents (mothers and fathers combined). Circles represent individual animals and bars represent medians. Mice had significantly higher latency to approach the ball containing the non-kin pup on PPD 28 than on PPD 16 ($p < 0.005$). Data were used only from mice that had data available from all four test days (n = 7 mothers, 8 fathers).

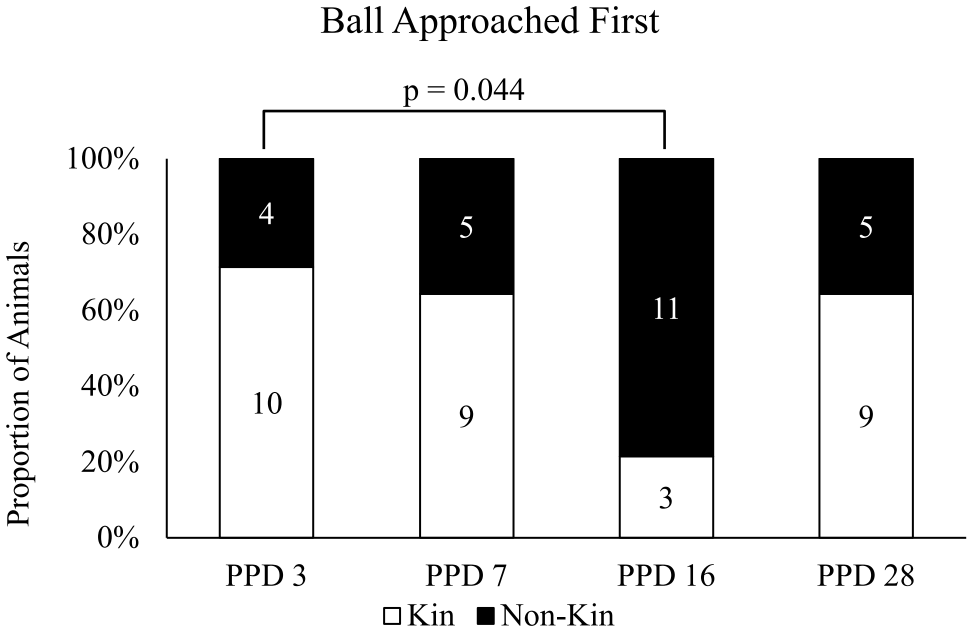

Figure 1.3. Proportion of California mouse parents (mothers and fathers combined) that approached the kin and non-kin ball first. Number inside each bar indicates the number of animals that approached the respective ball first. Significantly more animals approached the non-kin ball before the kin ball on PPD 16 than on PPD 3 (p = 0.044). Data were used only from mice that had data available from all four test days (n = 6 mothers, 8 fathers).

Chapter 2

Effects of age at first fatherhood on parental behavior and offspring outcomes in the biparental California mouse

Abstract

According to life history theory, older animals should invest more in current reproduction than younger animals because the likelihood of future reproduction decreases with age. Therefore, older parents may provide more and/or higher-quality parental care than younger parents. However, the ability of older animals to provide parental care may decline due to physiological senescence. We examined how fathers' age at first paternity affects biparental care and offspring in the biparental California mouse (*Peromyscus californicus*). We paired reproductively naïve males with 7-month-old females in either early (4 months) or late (16 months) adulthood. Age did not affect males' responses to unfamiliar pups shortly before pairing, days from pairing until parturition, litter composition, or pup development. We also compared fathers' behavior in an open field before and after the introduction of a pup into the arena. When alone, old fathers travelled longer distances than young fathers, but no age effects were observed in behavior towards pups. In observations of undisturbed families in their home cages, we found few differences in parental and non-parental behaviors between old and young fathers and their mates. However, mates of young males nursed their pups more than those of old males. Our results suggest little effect of age on paternal behavior in both sexually naïve and breeding males, within the age range studied.

1. Introduction

Life history theory predicts that in iteroparous species, older individuals should invest more in current reproduction than younger individuals due to the decline in residual reproductive value with age; as organisms age, the likelihood of future reproduction decreases (Fisher, 1930). In many species, one form of investment in current reproductive effort is parental care (Clutton-Brock, 1991). Thus, older parents are predicted to display more and/or higher-quality parental care towards offspring than younger parents. However, physiological senescence due to advanced age may negatively affect neuroendocrine processes underlying parental care as well as the physical capability to provide care (Lemaître & Gaillard, 2017). For example, older animals may be less efficient foragers than younger animals (Lecomte et al., 2010) or have impaired immune function due to age (Vleck, Vleck, & Palacios, 2011), potentially hindering older parents' ability to care for offspring.

Previous studies comparing caregiving behaviors of old and young parents have yielded conflicting results. One potential reason is that these studies have often compared older animals that have previously bred to younger, first-time parents, such that age is confounded with parity and experience (Benowitz, Head, Williams, Moore, & Royle, 2013; Cameron et al., 2000; Ericsson, Wallin, Ball, & Broberg, 2001; Reale & Bousses, 1995; Sasvári, Hegyi, Csörgõ, & Hahn, 2000; Wilcoxen, Boughton, & Schoech, 2010). However, studies that controlled for parity have generally supported the prediction that older parents provide greater amounts of care than younger parents. For example, in a study of Mongolian gerbils (*Meriones unguiculatus*), older mothers were more likely to retrieve and spend time nursing or in physical contact with their offspring, associated with faster pup growth, compared to younger mothers (Clark, Moghaddas, & Galef, 2002). Similarly, in the biparental (i.e., both parents provide care to offspring) burying beetle (*Nicrophorus vespilloides),* older mothers provide longer total durations of care during acute observations compared to younger mothers (Houslay, Kitchener, & Royle, 2020). In the same species, older fathers were found to continue care until offspring were older, compared to younger fathers (Benowitz et al., 2013).

The ages of both parents can influence not only the quality and amount of parental care but also the survival and quality of offspring. These effects may be sex-biased, with the age of one parent having a greater impact than that of the other (Angell, Janacek, & Rundle, 2022; Cholewa et al., 2021; Fay, Barbraud, Delord, & Weimerskirch, 2016; Lemaître & Gaillard, 2017; Sparkman et al., n.d.; Tidière et al., 2018). For example, in biparental species, the amount of investment by one parent may be influenced by the perceived attractiveness of its mate (Burley, 1988). In species in which females prefer older mates, mothers mated to older males might provide more parental care than those mated to younger males (Johnson & Gemmell, 2012; Monaghan, Maklakov, & Metcalfe, 2020). Moreover, mothers may behave more maternally to compensate if their mates provide inadequate care (reviewed in Houston, Székely, & McNamara, 2005). Therefore, if paternal age influences paternal care and/or attractiveness of fathers, we may expect mothers to alter their care of offspring depending on their mate's age. However, the effects of paternal age on parental behavior and reproductive outcomes have received little attention, especially in mammals (Roff, 1992).

To further our understanding of age effects in mammalian fathers, we examined how fathers' age at first reproduction affects biparental care and offspring outcomes in the California mouse (*Peromyscus californicus*). This species is socially monogamous in both captive and wild settings (Gubernick, 1988; Gubernick & Addington, 1994; Gubernick & Nordby, 1993), and mothers and fathers provide the same caregiving behaviors, except that only mothers nurse young (Gubernick & Alberts, 1987; Ribble, 1991). We hypothesized that although older fathers would produce pups that are lower in quality when young, old fathers would provide more paternal care than young fathers, resulting in equal pup quality between old and young fathers. However, testosterone influences paternal behavior in California mice (Trainor & Marler, 2001), and its production declines with age in rodents (Hardy & Schlegel, 2004). Therefore, it is possible that older fathers may instead provide less care than younger fathers.

2. Methods

2.1. Animals –

All animals were descended from California mice purchased from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC, USA) and were bred and housed at the University of California, Riverside (UCR). Mice were housed in polycarbonate cages (44 x 24 x 20 cm) under a 14:10 light:dark cycle (lights on at 05:00 h, lights off at 19:00 h) at approximately 22°C and 60-70% humidity. Aspen shavings and cotton were used for bedding and nesting material, respectively. Animals were provided food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA) and water ad lib. Animal health and births were checked twice each day, and cages were changed weekly.

Mice were removed from their natal families at 27-31 days of age, prior to the birth of younger siblings, and housed in groups of 2-4 same-sex animals until they were paired with a mate. In the wild, California mice can live at least 18 months, and approximately 40% of animals survive past their first year (McCabe & Blanchard, 1950; Merritt, 1978; Ribble, 1992b). Sexual maturity occurs by 3 months (Gubernick, 1988; Gubernick & Laskin, 1994; Gubernick & Nordby, 1992), but wild animals typically do not breed until approximately 8 months (Ribble, 1990). Therefore, we randomly assigned males to be paired with females at either 16 months (old males) or 4 months of age (young males). When they reached the appropriate ages, males were pair-housed with a female (~ 7 months of age) to whom they were no more closely related than first cousins. Males were weighed immediately before pairing, and all animals were subsequently weighed twice per week until parturition to monitor pregnancy in females and to habituate the animals to handling. A total of 49 pairs (24 old-male and 25 young-male) were formed. A timeline of the experimental design is provided in Figure 1.

2.2. Parental-behavior Test –

One week before forming breeding pairs, we tested each male's response to an unfamiliar pup between 10:00 and 12:00h. First, the male was placed in a clean cage identical to the home cage, containing a thin layer of fresh bedding, for a 15-minute habituation period, after which an unrelated, 2- to 5-day-old pup was introduced into the cage. The animals were observed and video-recorded for 1 hour unless the pup was attacked, in which case the test was terminated immediately and the pup was euthanized. Animals were returned to their home cages after the test. A trained observer, blind to condition (i.e., old versus young), used Behavioral Observation Research Interactive Software (BORIS, Friard & Gamba, 2016) to score the latency to approach the pup, latency to initiate parental behavior (i.e. grooming, huddling over, or carrying the pup), and durations of huddling over the pup, licking/grooming the pup, nestbuilding, general locomotion, autogrooming, and inactivity.

2.3. Postpartum Behavioral Observations –

Starting on the second day after the birth of the first litter (postpartum day [PPD] 2), we video-recorded each family under undisturbed conditions in their home cage three times per week until the pups reached weaning age (PPD 28). Each week, families were recorded for 4 h once at each of three times of day: 10:00 – 14:00h (lights on), 17:00 – 21:00h (transition from lights on to lights off), and 03:00 – 07:00h (transition from lights off to lights on). For observations during the dark phase of the light cycle, a red lamp was placed at each end of the cage for illumination.

Behaviors of both parents were scored by trained observers who were blind to the male's age group, using instantaneous sampling in BORIS. Approximately one third of the videos were scored at 15-minute intervals; however, for logistical reasons, we scored the remaining videos at 20-minute intervals. To account for this difference in sampling frequency, we analyzed the data as percent of total scan samples. At each scan, we recorded which of the following behaviors each parent was engaged in: passive nursing (mothers only), huddling over at least one pup (fathers only), grooming pups, nestbuilding, retrieving pups, in physical contact with at least one pup (excluding passive nursing, grooming, huddling, and retrieving), locomotion, eating, drinking, jumping, resting, autogrooming, or in physical contact with the mate but not pups. All behaviors, with the exception of the pairmates being in physical contact with each other, were scored as mutually exclusive. We scored mothers as “passive nursing” if any pups’ faces were in contact with the mother’s ventrum without her engaging in other behaviors; however, we recognize that the pups may have been attached to and possibly nursing from their mother while she performed other scored behaviors. In this species, pups tenaciously attach themselves to their mother’s nipples and are not easily dislodged, even while she is moving (Gilbert, 1995).

2.4. Open-field Pup Test –

The open-field test takes advantage of rodents’ aversion to open spaces and is a common method for measuring anxiety-like behavior (Ohl, 2003). By combining the open-field and parental-behavior tests, we can assess both anxiety-like behavior and responses to a pup under anxiogenic conditions (Perea-Rodriguez, Zhao, Harris, Raqueno, & Saltzman, 2018).

On PPD 11, between 10:00 and 12:00, a father was placed in an acrylic open-field arena (1m x 1m x 0.4m), illuminated from above by two lamps at 1400 lx (Perea-Rodriguez et al., 2018), for 10 minutes. A 2- to 5-day-old unfamiliar pup was then placed in the center of the open field for an additional 10 minutes; the test was terminated immediately if the pup was attacked. Both portions of the test were video recorded from above. For the first half of the test, the duration of time the animal spent in the center 50% of the field, the duration of time spent along the edges (i.e., outer 50%) of the field, the number of entries into and out of the center, and total distance traveled were scored using TopScan video-tracking software (CleverSys, Reston, VA, USA). For the second half of the test, latency to approach the pup, latency to contact the pup, and durations of contact with the pup, huddling over the pup, carrying the pup, inactivity, and general movement were scored by a trained observer, blind to paternal age, using BORIS.

2.5. Pup Development –

To compare pup development between litters from old and young fathers, pups were weighed once per week at 6- to 7- day intervals from PPD 3 through PPD 28 (weaning age). On PPD 28, we also characterized body composition (lean and fat mass) using MRI (EchoMRI-100, Echo Medical Systems, Houston, TX, USA; Zhao et al., 2017). This is a non-invasive procedure that does not require sedation or anesthesia and lasts approximately 2 minutes. Starting at PPD 11, pups were checked daily for eye opening.

2.6. Analysis –

Due to technical issues, approximately one quarter of the home-cage observations could not be scored. We accounted for this by performing multiple imputation by predictive mean matching based on male age and observation week using the *mice* (van Buuren & Groothuis-Oudshoorn, 2011) and *miceadds* (Robitzsch, Grund, Henke, & Robitzsch, 2017) packages in R (v.4.2.3, R Core Team, 2023). For the postpartum period, we compared home-cage behavior of both parents between old-male and young-male pairs using generalized linear mixed models (GLMM) using the *glmmtmb* (Brooks et al., 2017) package in R . We chose beta regression GLMMs because our data consisted of proportions between 0 and 1. To account for the zeros in our data from animals that were not observed performing a given behavior during a particular observation period, we included zero-inflated components in our models. For all models, we included male age and week of observation as fixed effects and pair ID as a random effect. We verified model fit, normality of residuals, and heterogeneity of variances using the *DHARMa* package in R (Hartig & Hartig, 2017). Post-hoc analyses were performed using ANOVAs and pairwise comparisons from the *car* package (Fox & Weisberg, 2011). Data from mothers and fathers were analyzed separately, as were data from the 17:00 – 21:00h (transition from lights on to lights off) and 03:00 – 07:00h (transition from lights off to lights on) observation periods. When scoring the observations conducted at 10:00 – 14:00h, we noticed that most animals spent nearly the entire period resting. We therefore excluded these observations from our analyses.

We compared offspring survival and development between old-male and young-male pairs using Mann-Whitney U tests. We also used Mann-Whitney tests to compare behavior of old and young males in the parental-behavior tests and the open-field pup test. To compare the body masses of old and young males prior to being paired with a mate, we transformed the data logarithmically to achieve normality and performed an independent samples t-test. All statistical analyses were interpreted using a critical P value of 0.05 (2-tailed).

3. Results

3.1. Male Body Mass

Immediately before being paired with females, old males (log body mass, 1.72 ± 0.02g) had significantly higher body mass than young males (log body mass, 1.59 ± 0.09g , t-test, $T = 4.867$, $p < 0.001$). We did not weigh fathers following the birth of their offspring to avoid handling animals more than necessary (Fig. 2).

3.2. Reproductive Outcomes

Eighteen of the 24 old-male and 18 of the 25 young-male pairs produced pups within 40 days after being paired. In three old-male pairs and 6 young-male pairs, the entire first litter died within the first week after parturition with no obvious cause of death. Of the pairs that produced a litter, 15 old-male pairs and 15 young-male pairs produced a second litter. The entire second litters of four old-male pairs and two young-male pairs died within the first week after parturition with no apparent cause of death. We did not find any significant differences between old-male and young-male pairs in the timing from pairing to the birth of the first litter, pup survival past the first week in either the first or the second litter, size of either litter, interbirth interval, or litter sex ratio of first litters (Mann-Whitney U tests, Table 1). We did not determine the sexes of pups in second litters.

3.3. Pup Development

We performed nested ANOVAS to analyze pups' weekly body mass across the 4-week pup-rearing period as well as fat and lean mass at weaning age. The proportion of male pups in each litter was used as a covariate because in this species, female pups tend to weigh more than male pups (Cantoni, Glaizot, & Brown, 1999). On PPD 28, per-pup body mass was significantly higher in litters of old fathers than those of young fathers ($F = 4.59$, $p = 0.037$); however, this difference was no longer significant when corrected for the proportion of male pups ($F = 2.47$, $p = 0.095$). Finally, all pups within a litter opened their eyes on the same day, and age at which the pups first opened their eyes did not differ between pups of old and young males (Table 2).

3.4. Parental-behavior Test

In the parental-behavior tests, conducted one week prior to pair formation, the proportion of males that performed parental behavior, attacked the pup, or neither did not differ significantly between old and young males (Chi-square, $p > 0.05$). Thirteen of the 24 sexually naive old males performed parental behavior (i.e., huddling, licking, and/or nest building), 6 attacked the pup, and 5 performed neither parental nor aggressive behavior. Among the 25 sexually naive young males, 12 responded parentally, 3 attacked, and 10 were neither parental nor aggressive.

Old and young males did not differ significantly in their latencies to touch or initiate parental behavior towards the pup, although old males tended to sniff the pup more quickly than young males (Mann-Whitney, $U = 395.50$, $p = 0.056$; Table 3). The two groups also did not differ in the duration of any pup-directed behaviors or in the total time spent in parental behavior. Young males spent more time resting without any movement than old males (Mann-Whitney, $U = 422.00$, $p = 0.015$, Table 3); no other non-pup-directed behaviors differed between the groups.

3.5. Open-field Pup Test

In the first 10 minutes of the open-field pup test (PPD 11), prior to introduction of the pup, old fathers traveled longer distances in both the outer half (Mann-Whitney, $U = 56.00$, $p = 0.011$) and the center half (Mann-Whitney, $U = 59.00$, $p = 0.016$) of the arena. Old and young fathers did not differ in the duration of time spent in either the outer or center half. We found no significant differences between age groups in either pup-directed or non-pup-directed behavior during the second half of the test, following introduction of the pup (Table 4).

3.6. Home-cage Behaviors

At 17:00 – 21:00h (transition from lights on to lights off), we observed significant effects of postpartum week (Type III Wald χ^2 test, $\chi^2 = 18.77$, $p < 0.001$) and a significant interaction between postpartum week and paternal age (Type III Wald χ^2 test, $\chi^2 = 12.73$, $p = 0.005$) in the number of scan samples in which fathers huddled over their pups (Fig. 3). Young fathers, but not old fathers, huddled over pups more often during postpartum week 1 than during postpartum weeks 3 (Tukey HSD, $p = 0.030$) and 4 (Tukey HSD, $p = 0.002$). During postpartum week 2, old fathers huddled over pups more frequently than old (Tukey HSD, $p = 0.008$) or young fathers during postpartum week 4 (Tukey HSD, $p = 0.006$). Among mothers, mates of young fathers showed a non-significant tendency to eat more than mates of old fathers (Type III Wald χ^2 test, $\chi^2 = 3.52$, $p = 0.061$). We did not find any other significant differences between old and young fathers or their mates (Table 5).

At 03:00 – 07:00h (transition from lights off to lights on), we found a marginally significant interaction between paternal age and postpartum week in physical contact between mates (Type III Wald χ^2 test, $\chi^2 = 7.69$, $p = 0.053$): young fathers tended to be in contact with their mates more during postpartum week 2 than postpartum week 4, but this was not observed in old fathers (Fig. 4). We additionally observed that mothers mated to young fathers spent significantly more time nursing their pups compared to mothers mated to old fathers (Type III Wald χ^2 test, $\chi^2 = 5.359$, $p = 0.021$, Fig. 5). Duration of nursing also showed a marginally significant interaction between paternal age and postpartum week (Type III Wald χ^2 test, $\chi^2 = 7.726$, $p = 0.052$). However, post-hoc tests did not reveal any significant pairwise differences across weeks for either group analyzed separately or between groups at any given time point. Finally, we found a significant interaction between paternal age and postpartum week in the frequency of eating observed in mothers (Type III Wald χ^2 test, $\chi^2 = 11.681$, $p = 0.009$), as well as significant main effect of paternal age (Type III Wald χ^2 test, $\chi^2 = 11.106$, $p = 0.011$) and postpartum week (Type III Wald χ^2 test, $\chi^2 = 7.742$, $p = 0.005$). During postpartum week 1, mates of young fathers ate more compared to mates of old fathers during postpartum weeks 2 (Tukey HSD, $p = 0.020$) and 3 (Tukey HSD, $p = 0.018$, Fig. 6). No other significant effects of paternal age or postpartum week were observed at this time of day (Table 6).

4. Discussion

In this study, we examined the behavioral and reproductive consequences of delayed reproduction in male California mice to test the hypothesis that old first-time fathers provide more care to their offspring than young first-time fathers. However, we found little evidence of differences in paternal behavior between old and young fathers, and the few significant effects of paternal age that we did observe suggest that old fathers may provide less, rather than more, paternal care than young fathers. We also observed that mates of young fathers nursed their pups more than those of old fathers, but no other differences in maternal behavior.

4.1.1. Effects of Paternal Age on Paternal Behavior

We did not find any significant differences between the pup-directed behaviors of old and young fathers during the home-cage observations. Thus, our results do not support life history theory's prediction that older parents will invest more in offspring (i.e., provide parental care) than young parents. We previously found a similar lack of differences in pup-directed behaviors between old and young sexually naïve adult males; however, the age difference between old and young males in that study (266.4 ± 4.1 vs 136.8 ± 2.3 days respectively; Nguyen, Zhao, & Saltzman, 2020) was smaller than in the present study (Old males: 481.0 ± 0.841 days, Young males: 117.0 ± 1.07). Similarly, Kenkel et al. (2019) found no effects of age on paternal behavior in prairie voles. In that study, however, all males had previously sired several litters, whereas in our study, males in both age groups were first-time fathers.

Prior to being housed with a female, old and young males in the present study showed no significant differences in pup-directed behaviors; however, old sexually naïve males tended to sniff unfamiliar pups in paternal-behavior tests sooner than young males. This difference might indicate age effects on exploration, anxiety-like behavior, and/or neophobia. Old sexually naïve males in our study also spent less time resting without movement than young males during the parental-behavior test. In some strains of house mice, older adults are more exploratory in novel environments than younger adults (Miyamoto, Kiyota, Nishiyama, & Nagaoka, 1992). Additionally, old fathers in our study traveled more in an empty open field than young fathers, indicating lower levels of anxiety-like behaviors and/or higher levels of exploratory behavior in the former.

Similarly, Kenkel et al. (2019) observed that older prairie vole fathers displayed fewer anxiety-like behaviors in an open field. Our lab previously found greater sensitivity to dexamethasone-induced negative feedback on the hypothalamic-pituitary-adrenal (HPA) axis, but no other differences, in old versus young sexually naïve adult male California mice, indicating that the HPA axis is more easily suppressed in older males than younger males (Harris & Saltzman, 2013). It is possible, therefore, that the behavioral differences between old and young males in the present study may be mediated by differences in HPA activity. Considering that both the cage in the parental behavior test and the open-field arena are novel environments, a potential stressor, the present findings suggest that male California mice become more exploratory and/or less anxious with age across adulthood.

One possible reason why we observed few differences between old and young fathers is that, although we selected ecologically relevant ages for old and young males, our old fathers may not have yet reached a senescent age. California mice can live at least 5 years in captivity (Havighorst, Crossland, & Kiaris, 2017); therefore, we might see stronger effects of paternal age if we repeated this study using even older males.

4.1.2. Effects of Paternal Age on Maternal Behavior

Females mated to young fathers ate more frequently than those mated to old fathers. Mates of young fathers also nursed their pups more frequently than mates of old fathers during the transition between the dark and light phase. Possibly, mothers may have adjusted the amount of time spent passively nursing in response to mate quality;

younger males being perceived as higher or lower quality than older males (Mashoodh, Franks, Curley, & Champagne, 2012; Sheldon, 2000). However, in the absence of differences between the paternal behaviors of old and young fathers or in body mass and body composition of their pups, we do not have sufficient evidence to conclude that mothers responded to any potential effects of paternal age on mate quality.

4.2 Effects of Paternal Age on Reproductive and Offspring Outcomes

We found no differences in reproductive outcomes (e.g., litter size, litter sex ratio, interbirth interval, pup survival) between old and young fathers. These results are consistent with those of previous studies in other rodents (Caballero-Campo et al., 2018; García-Palomares, Pertusa, et al., 2009); however, Caballero-Campos et al (2018) found that time to conception increases with paternal age in house mice.

We found no effects of paternal age on reproduction or offspring growth and development. This might be because our study was conducted in a captive setting, in which animals were provided food and water ad lib, temperature was held constant within the animals' thermoneutral zone, and mice did not have to compete with conspecifics, defend territories, or evade predators. Our results align with those of previous studies in California mice that suggest fathers might increase offspring survival and accelerate offspring development only in natural environments or in energetically challenging captive conditions, such as when mice are housed at low temperatures or are required to work for food (Dudley, 1974; Gubernick & Teferi, 2000; Vieira & Brown, 2003; Wright & Brown, 2002).

We did not examine the behavior or reproductive success of offspring. In other rodents, advanced paternal age has detrimental effects on offspring’s motor development (García-Palomares, Pertusa, et al., 2009), learning ability (Auroux, 1983; García-Palomares, Pertusa, et al., 2009), social and exploratory behavior (R. G. Smith et al., 2009), reproduction (Caballero-Campo et al., 2018; García-Palomares, Navarro, et al., 2009), and lifespan (García-Palomares, Navarro, et al., 2009; Xie et al., 2018). Furthermore, in other rodents, pups that receive higher levels of caregiving from mothers (Champagne, 2008; Stolzenberg & Champagne, 2016) or fathers (Ahern et al., 2010; Gromov, 2009; Jia, Tai, An, & Zhang, 2011) provide more caregiving, as adults, to their own offspring. In human and non-human animals, age-related changes to the germ line mediate many of the effects of advanced paternal age on offspring behavior and health (reviewed in Carrageta et al., 2022 and Momand, Xu, & Walter, 2013). Therefore, although we did not observe differences in paternal behavior or reproductive outcomes between old and young fathers, advanced paternal age might have long-term consequences for offspring into adulthood.

Overall, our findings do not support our hypothesis that older first-time fathers provide more paternal care compared to younger first-time fathers. Instead, our results suggest little effect of age on paternal behavior in both sexually naïve and breeding male California mice, and only small effects on maternal behavior. It is possible, however, that additional studies using old and young males with a greater age difference, or experiments examining mice under environmentally challenging conditions, might reveal more pronounced effects of paternal age on parental care, maternal care, and/or reproductive outcomes.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2010). Parental Division of Labor , Coordination , and the Effects of Family Structure on Parenting in Monogamous Prairie Voles (Microtus ochrogaster). https://doi.org/10.1002/dev.20498

Angelier, F., Shaffer, S. A., Weimerskirch, H., & Chastel, O. (2006). Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird: The wandering albatross. *General and Comparative Endocrinology*, *149*(1), 1–9. https://doi.org/10.1016/j.ygcen.2006.04.006

Angell, C. S., Janacek, R., & Rundle, H. D. (2022). Maternal and Paternal Age Effects on Male Antler Flies: A Field Experiment. *American Naturalist*, *199*(3), 436–442. https://doi.org/10.1086/718236

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology*, *14*(4), 451–479.

Auroux, M. (1983). Decrease of learning capacity in offspring with increasing paternal age in the rat. *Teratology*, *27*(2), 141–148. https://doi.org/10.1002/tera.1420270202

Bales, K. L., & Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Hormones and Behavior*, *77*, 249–259. https://doi.org/10.1016/j.yhbeh.2015.05.021

Benowitz, K. M., Head, M. L., Williams, C. A., Moore, A. J., & Royle, N. J. (2013). Male age mediates reproductive investment and response to paternity assurance. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1764), 20131124. https://doi.org/10.1098/rspb.2013.1124

Bester-Meredith, J. K., Burns, J. N., Conley, M. F., Mammarella, G. E., & Ng, N. D. (2017). Peromyscus as a model system for understanding the regulation of maternal behavior. *Seminars in Cell and Developmental Biology*, *61*, 99–106. https://doi.org/10.1016/j.semcdb.2016.07.001

Birnie, A. K., Taylor, J. H., Cavanaugh, J., & French, J. A. (2013). Quality of maternal and paternal care predicts later stress reactivity in the cooperatively-breeding marmoset (Callithrix geoffroyi). *Psychoneuroendocrinology*, *38*(12), 3003–3014. https://doi.org/10.1016/j.psyneuen.2013.08.011

Boheemen, L. A. Van, Richardson, D. S., Burke, T., Komdeur, J., & Dugdale, H. L. (2019). Compensatory and additive helper effects in the cooperatively breeding Seychelles warbler (Acrocephalus sechellensis), (October 2018), 2986–2995. https://doi.org/10.1002/ece3.4982

Bolton, J. L., Molet, J., Ivy, A., & Baram, T. Z. (2017). New insights into early-life stress and behavioral outcomes. *Current Opinion in Behavioral Sciences*, *14*, 133–139. https://doi.org/10.1016/j.cobeha.2016.12.012

Braun, K., & Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *Journal of Neuroendocrinology*, *26*(10), 697–706. https://doi.org/10.1111/jne.12174

Bridges, R. S. (2016). Long-term alterations in neural and endocrine processes induced by motherhood in mammals. *Hormones and Behavior*, *77*, 193–203. https://doi.org/10.1016/j.yhbeh.2015.09.001

Brooks, M. E., Kristensen, K., Benthem, K. J. van, Magnusson, A., Berg, C. W., Nielsen, A., … Bolker, B. M. (2017). Modeling zero-inflated count data with glmmTMB. *BioRxiv*, 132753. Retrieved from https://www.biorxiv.org/content/10.1101/132753v1%0Ahttps://www.biorxiv.org/content/10.1101/132753v1.abstract

Burley, N. (1988). The differential-allocation hypothesis: an experimental test. *American Naturalist*, *132*(5), 611–628. https://doi.org/10.1086/284877

Caballero-Campo, P., Lin, W., Simbulan, R., Liu, X., Feuer, S., Donjacour, A., & Rinaudo, P. F. (2018). Advanced Paternal Age Affects Sperm Count and Anogenital Distance in Mouse Offspring. *Reproductive Sciences*, *25*(4), 515–522. https://doi.org/10.1177/1933719118759441

Caldji, C., Diorio, J., & Meaney, M. J. (2000). Variations in maternal care in infancy regulate the development of stress reactivity. *Biological Psychiatry*, *48*(12), 1164–1174. https://doi.org/10.1016/S0006-3223(00)01084-2

Caldwell, H. K. (2018). Oxytocin and sex differences in behavior. *Current Opinion in Behavioral Sciences*, *23*, 13–20. https://doi.org/10.1016/j.cobeha.2018.02.002

Cameron, E. Z. (1998). Is suckling behavior a useful predictor of milk intake? A review. *Animal Behaviour*, *56*(3), 521–532. https://doi.org/10.1006/anbe.1998.0793

Cameron, E. Z., Linklater, W. L., Stafford, K. J., Minot, E. O. 0, Minot, E. O. 0, Cameron, E. Z., … Minot, E. O. 0. (2000). Aging and Improving Reproductive Success in Horses: Declining Residual Reproductive Value or Just Older and Wiser? *Source: Behavioral Ecology and Sociobiology Behav Ecol Sociobiol*, *47*(47), 243–249. Retrieved from http://www.jstor.org/stable/4601737

Cantoni, D., Glaizot, O., & Brown, R. E. (1999). Effects of sex composition of the litter on anogenital distance in California mice (Peromyscus californicus). *Canadian Journal of Zoology*, *77*(1), 124–131. https://doi.org/10.1139/cjz-77-1-124

Carrageta, D. F., Guerra-carvalho, B., Angélica, M., Marc, S., Oliveira, P. F., & Alves, M. G. (2022). Animal models of male reproductive ageing to study testosterone production and spermatogenesis, 1341–1360.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, *29*(3), 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Cholewa, M., Jankowiak, Ł., Szenejko, M., Dybus, A., Śmietana, P., & Wysocki, D. (2021). The effects of parental age difference on the offspring sex and fitness of European blackbirds. *PeerJ*, *9*, 1–20. https://doi.org/10.7717/peerj.10858

Clark, M. M., Moghaddas, M., & Galef, B. G. (2002). Age at first mating affects parental effort and fecundity of female Mongolian gerbils. *Animal Behaviour*, *63*(6), 1129–1134. https://doi.org/10.1006/anbe.2002.3019

Clutton-brock, T. H. (2004). Cooperative breeding in mammals.

Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, New Jersey, USA: Princeton University Press.

Creel, S., Dantzer, B., Goymann, W., & Rubenstein, D. R. (2013). The ecology of stress: Effects of the social environment. *Functional Ecology*, *27*(1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x

Crick, H. Q. P. (1992). Load-lightening in cooperatively breeding birds and the cost of reproduction. *Ibis*, *134*(1), 56–61. https://doi.org/10.1111/j.1474-919X.1992.tb07230.x

Dudley, D. (1974). Contributions of paternal care to the growth and development of the young in Peromyscus californicus. *Behavioral Biology*, *11*(2), 155–166. https://doi.org/10.1016/S0091-6773(74)90305-8

English, S. (2009). Individual variation in cooperative behaviour in meerkats, (October).

Ericsson, G., Wallin, K., Ball, J. P., & Broberg, M. (2001). Age-related reproductive effort and senescence in free-ranging moose, Alces alces. *Ecology*, *82*(6), 1613–1620. https://doi.org/10.1890/0012-9658(2001)082[1613:ARREAS]2.0.CO;2

Fay, R., Barbraud, C., Delord, K., & Weimerskirch, H. (2016). Paternal but not maternal age influences early-life performance of offspring in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828). https://doi.org/10.1098/rspb.2015.2318

Fisher, R. A. (1930). *The genetical theory of natural selection. The genetical theory of natural selection.* Oxford, England: Clarendon Press. https://doi.org/10.5962/bhl.title.27468

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Sage publications.

Francis, D. D., Champagne, F. A., Liu, D., & Meaney, M. J. (1999). Maternal care, gene expression, and the development of individual differences in stress reactivity. *Annals of the New York Academy of Sciences*, *896*, 66–84. https://doi.org/10.1111/j.1749-6632.1999.tb08106.x

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

García-Palomares, S., Navarro, S., Pertusa, J. F., Hermenegildo, C., García-Pérez, M. A., Rausell, F., … Tarín, J. J. (2009). Delayed Fatherhood in Mice Decreases Reproductive Fitness and Longevity of Offspring. *Biology of Reproduction*, *80*(2), 343–349. https://doi.org/10.1095/biolreprod.108.073395

García-Palomares, S., Pertusa, J. F., Miñarro, J., García-Pérez, M. A., Hermenegildo, C., Rausell, F., … Tarín, J. J. (2009). Long-Term Effects of Delayed Fatherhood in Mice on Postnatal Development and Behavioral Traits of Offspring. *Biology of Reproduction*, *80*(2), 337–342. https://doi.org/10.1095/biolreprod.108.072066

Gilbert, A. N. (1995). Tenacious nipple attachment in rodents: the sibling competition hypothesis. *Animal Behaviour*, *50*(4), 881–891. https://doi.org/10.1016/0003-3472(95)80091-3

González-Mariscal, G., Caba, M., Martínez-Gómez, M., Bautista, A., & Hudson, R. (2016). Mothers and offspring: The rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. *Hormones and Behavior*, *77*, 30–41. https://doi.org/10.1016/j.yhbeh.2015.05.011

Greenberg, G. D., van Westerhuyzen, J. A., Bales, K. L., & Trainor, B. C. (2012). Is it all in the family? The effects of early social structure on neural-behavioral systems of prairie voles (Microtus ochrogaster). *Neuroscience*, *216*, 46–56. https://doi.org/10.1016/j.neuroscience.2012.04.063

Gromov, V. S. (2009). Interactions of partners in family pairs, care of the offspring, and the role of tactile stimulation in formation of parental behavior of the Mongolian gerbil (Meriones unguiculatus) under laboratory conditions. *Biology Bulletin*, *36*(5), 479–488. https://doi.org/10.1134/s1062359009050082

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. https://doi.org/10.2307/1381649

Gubernick, D. J., & Addington, R. L. (1994). The stability of female social and mating preferences in the monogamous California mouse, peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1079

Gubernick, D. J., & Alberts, J. R. (1987). The biparental care system of the California mouse, Peromyscus californicus.. *Journal of Comparative Psychology*, *101*(2), 169–177. https://doi.org/10.1037/0735-7036.101.2.169

Gubernick, D. J., & Laskin, B. (1994). Mechanisms influencing sibling care in the monogamous biparental California mouse, Peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1356

Gubernick, D. J., & Nordby, J. C. (1992). Parental influences on female puberty in the monogamous California mouse, Peromyscus californicus. *Animal Behaviour*, *44*(PART 2), 259–267. https://doi.org/10.1016/0003-3472(92)90032-5

Gubernick, D. J., & Nordby, J. C. (1993). Mechanisms of sexual fidelity in the monogamous California mouse, Peromyscus californicus. *Behavioral Ecology and Sociobiology*, *32*(3), 211–219. https://doi.org/10.1007/BF00173779

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *The Royal Society*, *267*(1439), 147–150. https://doi.org/10.1098/rspb.2000.0979

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Hardy, M. P., & Schlegel, P. N. (2004). Testosterone Production in the Aging Male: Where Does the Slowdown Occur? *Endocrinology*, *145*(10), 4439–4440. https://doi.org/10.1210/en.2004-0888

Harper, L. V. (1981). Offspring effects upon parents. In *Parental care in mammals* (pp. 117–177). Springer.

Harris, B. N., & Saltzman, W. (2013). Effects of aging on hypothalamic-pituitary-adrenal (HPA) axis activity and reactivity in virgin male and female California mice (Peromyscus californicus). *General and Comparative Endocrinology*, *186*, 41–49. https://doi.org/10.1016/j.ygcen.2013.02.010

Harrison, F., Barta, Z., Cuthill, I., & Székely, T. (2009). How is sexual conflict over parental care resolved? A meta-analysis. *Journal of Evolutionary Biology*, *22*(9), 1800–1812. https://doi.org/10.1111/j.1420-9101.2009.01792.x

Hartig, F., & Hartig, M. F. (2017). Package 'DHARMa.' *R Package*.

Havighorst, A., Crossland, J., & Kiaris, H. (2017). Peromyscus as a model of human disease. *Seminars in Cell and Developmental Biology*, *61*, 150–155. https://doi.org/10.1016/j.semcdb.2016.06.020

Heinsohn, R., & Legge, S. (1999). The cost of helping. *Trends in Ecology and Evolution*, *14*(2), 53–57. https://doi.org/10.1016/S0169-5347(98)01545-6

Houslay, T. M., Kitchener, P. A., & Royle, N. J. (2020). Are older parents less flexible? Testing age-dependent plasticity in Nicrophorus vespilloides burying beetles. *Animal Behaviour*, *162*, 79–86. https://doi.org/https://doi.org/10.1016/j.anbehav.2020.01.012

Houston, A. I., Székely, T., & McNamara, J. M. (2005). Conflict between parents over care. *Trends in Ecology and Evolution*, *20*(1), 33–38. https://doi.org/10.1016/j.tree.2004.10.008

Hudson, R., Bautista, A., Reyes-Meza, V., Montor, J. M., & Rödel, H. G. (2011). The effect of siblings on early development: A potential contributor to personality differences in mammals. *Developmental Psychobiology*, *53*(6), 564–574. https://doi.org/10.1002/dev.20535

Hudson, R., & Trillmich, F. (2008). Sibling competition and cooperation in mammals: Challenges, developments and prospects. *Behavioral Ecology and Sociobiology*, *62*(3), 299–307. https://doi.org/10.1007/s00265-007-0417-z

Jašarević, E., Williams, S. A., Roberts, R. M., Geary, D. C., & Rosenfeld, C. S. (2012). Spatial navigation strategies in Peromyscus: A comparative study. *Animal Behaviour*, *84*(5), 1141–1149. https://doi.org/10.1016/j.anbehav.2012.08.015

Jia, R., Tai, F., An, S., & Zhang, X. (2011). Neonatal paternal deprivation or early deprivation reduces adult parental behavior and central estrogen receptor α expression in mandarin voles (Microtus mandarinus). *Behavioural Brain Research*, *224*(2), 279–289. https://doi.org/10.1016/j.bbr.2011.05.042

Johnson, S. L., & Gemmell, N. J. (2012). Are old males still good males and can females tell the difference?: Do hidden advantages of mating with old males off-set costs related to fertility, or are we missing something else. *BioEssays*, *34*(7), 609–619. https://doi.org/10.1002/bies.201100157

Johnstone, R. A., & Hinde, C. A. (2006). Negotiation over offspring care — how should parents respond to each other' s efforts ?, (July). https://doi.org/10.1093/beheco/arl009

Jurkevich, A., Grossmann, R., Balthazart, J., & Viglietti-Panzica, C. (2001). Gender-related changes in the avian vasotocin system during ontogeny. *Microscopy Research and Technique*, *55*(1), 27–36. https://doi.org/10.1002/jemt.1153

Kenkel, W. M., Paredes, J., Yee, J. R., Bales, K. L., & Carter, C. S. (2012). Neuroendocrine and Behavioural Responses to Exposure to an Infant in Male Prairie Voles Neuroendocrinology, (12), 874–886. https://doi.org/10.1111/j.1365-2826.2012.02301.x

Komdeur, J., & Ekman, J. (2010). Adaptations and constraints in the evolution of delayed dispersal: implications for cooperation. *Social Behaviour: Genes, Ecology and Evolution. Cambridge Univ. Press, Cambridge*, 306–327.

Kross, S. M., & Nelson, X. J. (2013). Factors influencing the behavioural development of juvenile New Zealand Falcons (Falco novaeseelandiae). *Emu*, *113*(1), 84–87. https://doi.org/10.1071/MU12020

Lecomte, V. J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., … Chastel, O. (2010). Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Sciences*, *107*(14), 6370–6375. https://doi.org/10.1073/pnas.0911181107

Lemaître, J. F., & Gaillard, J. M. (2017). Reproductive senescence: new perspectives in the wild. *Biological Reviews*, *92*(4), 2182–2199. https://doi.org/10.1111/brv.12328

Lonstein, J. S., & De Vries, G. J. (2000). Sex differences in the parental behavior of rodents. *Neuroscience and Biobehavioral Reviews*, *24*(6), 669–686. https://doi.org/10.1016/S0149-7634(00)00036-1

Mashoodh, R., Franks, B., Curley, J. P., & Champagne, F. A. (2012). Paternal social enrichment effects on maternal behavior and offspring growth. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(SUPPL.2), 17232–17238. https://doi.org/10.1073/pnas.1121083109

McCabe, T. T., & Blanchard, B. D. (1950). Three species of Peromyscus.

Merritt, J. F. (1978). Peromyscus californicus. *Mammalian Species*, (85), 1. https://doi.org/10.2307/3503909

Miyamoto, M., Kiyota, Y., Nishiyama, M., & Nagaoka, A. (1992). Senescence-Accelerated Mouse (SAM): Age-Related Reduced Anxiety-Like Behavior in the SAM-P / 8 Strain, *51*(17), 979–985.

Momand, J. R., Xu, G., & Walter, C. A. (2013). Minireview The Paternal Age Effect : A Multifaceted Phenomenon, *88*(March), 1–9. https://doi.org/10.1095/biolreprod.112.103440

Monaghan, P., Maklakov, A. A., & Metcalfe, N. B. (2020). Intergenerational Transfer of Ageing: Parental Age and Offspring Lifespan. *Trends in Ecology and Evolution*, *35*(10), 927–937. https://doi.org/10.1016/j.tree.2020.07.005

Murgatroyd, C. A., Peña, C. J., Podda, G., Nestler, E. J., & Nephew, B. C. (2015). Early life social stress induced changes in depression and anxiety associated neural pathways which are correlated with impaired maternal care. *Neuropeptides*, *52*(3), 103–111. https://doi.org/10.1016/j.npep.2015.05.002

Nguyen, C. T. Y., Zhao, M., & Saltzman, W. (2020). Effects of sex and age on parental motivation in adult virgin California mice. *Behavioural Processes*, *178*(June), 104185. https://doi.org/10.1016/j.beproc.2020.104185

Ohl, F. (2003). Testing for anxiety. *Clinical Neuroscience Research*, *3*(4–5), 233–238. https://doi.org/10.1016/S1566-2772(03)00084-7

Ottinger, M. A. (2010). Mechanisms of reproductive aging: conserved mechanisms and environmental factors. *Annals of the New York Academy of Sciences*, *1204*(1), 73–81. https://doi.org/10.1111/j.1749-6632.2010.05653.x

Perea-Rodriguez, J. P., Zhao, M., Harris, B. N., Raqueno, J., & Saltzman, W. (2018). Behavioral and endocrine consequences of placentophagia in male California mice (Peromyscus californicus). *Physiology & Behavior*, *188*(November 2017), 283–290. https://doi.org/10.1016/j.physbeh.2018.02.022

Pike, K. N., Ashton, B. J., Morgan, K. V, & Ridley, A. R. (2019). Social and Individual Factors Influence Variation in Offspring Care in the Cooperatively Breeding Western Australian Magpie, *7*(April), 1–13. https://doi.org/10.3389/fevo.2019.00092

Pilakouta, N., Hanlon, E. J. H., & Smiseth, P. T. (2018). Biparental care is more than the sum of its parts: Experimental evidence for synergistic effects on offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884), 1–7. https://doi.org/10.1098/rspb.2018.0875

Pillay, N., & Rymer, T. L. (2015). Alloparenting enhances the emotional, social and cognitive performance of female African striped mice, Rhabdomys pumilio. *Animal Behaviour*, *99*, 43–52. https://doi.org/10.1016/j.anbehav.2014.10.003

R Core Team. (2023). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Reale, D., & Bousses, P. (1995). Effect of ewe age and high population density on the early nursing behaviour of mouflon. *Ethology Ecology and Evolution*, *7*(4), 323–334. https://doi.org/10.1080/08927014.1995.9522940

Reeve, H. K. (1992). Queen activation of lazy workers in colonies of the eusocial naked mole-rat, *358*(July), 147–149.

Ribble, D. O. (1990). *Population and social dynamics of the California mouse (Peromyscus californicus)*. University of California, Berkeley.

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 161–166. https://doi.org/10.1007/BF00166397

Ribble, D. O. (1992a). Dispersal in a monogamous rodent, Peromyscus californicus. *Ecology*, *73*(3), 859–866. https://doi.org/10.2307/1940163

Ribble, D. O. (1992b). Lifetime reproductive success and its correlates in the monogamous rodent, Peromyscus californicus. *Journal of Animal Ecology*, *61*(2), 457–468. https://doi.org/10.2307/5336

Ribble, D. O., & Salvioni, M. (1990). Social Organization and Nest Co-Occupancy in Peromyscus californicus, a monogamous rodent, 9–15.

Riedman, M. L. (1982). The Evolution of Alloparental Care and Adoption in Mammals and Birds. *The Quarterly Review of Biology*, *57*(4), 405–435. https://doi.org/10.1086/412936

Robitzsch, A., Grund, S., Henke, T., & Robitzsch, M. A. (2017). Package 'miceadds.' *Vienna: R Foundation for Statistical Computing*.

Rödel, H. G., Meyer, S., Prager, G., Stefanski, V., & Hudson, R. (2010). Litter size is negatively correlated with corticosterone levels in weanling and juvenile laboratory rats. *Physiology and Behavior*, *99*(5), 644–650. https://doi.org/10.1016/j.physbeh.2010.01.032

Rödel, H. G., Von Holst, D., & Kraus, C. (2009). Family legacies: Short- and long-term fitness consequences of early-life conditions in female European rabbits. *Journal of Animal Ecology*, *78*(4), 789–797. https://doi.org/10.1111/j.1365-2656.2009.01537.x

Rogers, F. D., Rhemtulla, M., Ferrer, E., & Bales, K. L. (2018). Longitudinal trajectories and inter-parental dynamics of prairie vole biparental care. *Frontiers in Ecology and Evolution*, *6*(JUN), 1–13. https://doi.org/10.3389/fevo.2018.00073

Rosenbaum, S., & Gettler, L. T. (2018). Physiology & Behavior With a little help from her friends (and family) part I : the ecology and evolution of non-maternal care in mammals. *Physiology & Behavior*, *193*(November 2017), 1–11. https://doi.org/10.1016/j.physbeh.2017.12.025

Royle, N. J., Smiseth, P. T., & Kölliker, M. (2012). *The evolution of parental care*. Oxford University Press.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neuroscience and Biobehavioral Reviews*, *26*(6), 665–678. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12479841

Salo, A. L., & French, J. A. (1989). Early experience, reproductive success, and development of parental behaviour in Mongolian gerbils. *Animal Behaviour*, *38*(4), 693–702. https://doi.org/10.1016/S0003-3472(89)80015-6

Sasvári, L., Hegyi, Z., Csörgõ, T., & Hahn, I. (2000). Age-dependent diet change, parental care and reproductive cost in tawny owls Strix aluco. *Acta Oecologica*, *21*(4–5), 267–275. https://doi.org/10.1016/S1146-609X(00)01080-8

Scheibler, E., Weinandy, R., & Gattermann, R. (2005). Intra-family aggression and offspring expulsion in Mongolian gerbils (Meriones unguiculatus) under restricted environments. *Mammalian Biology*, *70*(3), 137–146. https://doi.org/10.1016/j.mambio.2004.12.002

Sheldon, B. C. (2000). Differential allocation: Tests, mechanisms and implications. *Trends in Ecology and Evolution*, *15*(10), 397–402. https://doi.org/10.1016/S0169-5347(00)01953-4

Smith, P. T., Kolliker, M., & Royle, N. J. (2012). What is parental care. *The Evolution of Parental Care*, 1–14.

Smith, R. G., Kember, R. L., Mill, J., Fernandes, C., Schalkwyk, L. C., Buxbaum, J. D., & Reichenberg, A. (2009). Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: A mouse model. *PLoS ONE*, *4*(12), 4–8. https://doi.org/10.1371/journal.pone.0008456

Snowdon, C. T., & Pickhard, J. J. (1999). Family feuds: Severe aggression among cooperatively breeding cotton-top tamarins. *International Journal of Primatology*, *20*(5), 651–663. https://doi.org/10.1023/A:1020796517550

Snowdon, C. T., & Ziegler, T. E. (2007). Growing up cooperatively: family processes and infant care in marmosets and tamarins. *Journal of Developmental Processes*, *2*(1), 40–66.

Sparkman, A. M., Blois, M., Adams, J., Waits, L., Miller, D. A. W., & Murray, D. L. (n.d.). Evidence for sex-specific reproductive senescence in monogamous cooperatively breeding red wolves. https://doi.org/10.1007/s00265-016-2241-9

Stead, S., Mucha, S., & Bădescu, I. (2019). Alloparental Care. *Encyclopedia of Animal Cognition and Behavior*, (Wisenden 1999), 1–7. https://doi.org/10.1007/978-3-319-47829-6_1400-1

Stiver, K. A., & Alonzo, S. H. (2011). Alloparental care increases mating success. *Behavioral Ecology*, *22*(1), 206–211. https://doi.org/10.1093/beheco/arq186

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Hormones and Behavior*, *77*, 204–210. https://doi.org/10.1016/j.yhbeh.2015.07.005

Stone, A. I., Mathieu, D., Griffin, L., & Bales, K. L. (2010). Alloparenting experience affects future parental behavior and reproductive success in prairie voles (Microtus ochrogaster). *Behavioural Processes*, *83*(1), 8–15. https://doi.org/10.1016/j.beproc.2009.08.008

Storey, A. E., & Walsh, C. J. (2013). Biological basis of mammalian paternal behavior. *Handbook of Father Involvement: Multidisciplinary Perspectives*, *2*, 3–22.

Swartz, K. B., & Rosenblum, L. A. (1981). The social context of parental behavior. In *Parental care in mammals* (pp. 417–454). Springer.

Tidière, M., Thevenot, X., Deligiannopoulou, A., Douay, G., Whipple, M., Siberchicot, A., … Lemaître, J. F. (2018). Maternal reproductive senescence shapes the fitness consequences of the parental age difference in ruffed lemurs. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1886). https://doi.org/10.1098/rspb.2018.1479

Trainor, B. C., & Marler, C. A. (2001). Testosterone, paternal behavior, and aggression in the monogamous California mouse (Peromyscus californicus). *Hormones and Behavior*, *40*(1), 32–42. https://doi.org/10.1006/hbeh.2001.1652

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In R. L. Trivers (Ed.), *Sexual Selection and the Descent of Man* (pp. 136–179). Routledge. https://doi.org/10.4324/9781315129266-7

Trivers, R. L. (1974). Parent-Offspring Conflict. *American Zoologist*, *14*(1), 249–264. https://doi.org/10.1093/icb/14.1.249

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, *45*(3), 1–67. https://doi.org/10.18637/jss.v045.i03

Vleck, C. M., Vleck, D., & Palacios, M. G. (2011). Evolutionary ecology of senescence: A case study using tree Swallows, Tachycineta bicolor. *Journal of Ornithology*. https://doi.org/10.1007/s10336-010-0629-2

Wilcoxen, T. E., Boughton, R. K., & Schoech, S. J. (2010). Older can be better: Physiological costs of paternal investment in the Florida scrub-jay. *Behavioral Ecology and Sociobiology*, *64*(10), 1527–1535. https://doi.org/10.1007/s00265-010-0966-4

Wittenberger, J. F., & Tilson, R. L. (1980). The evolution of monogamy: hypotheses and evidence. *Annual Review of Ecology and Systematics*, *11*(1), 197–232.

Woodroffe, R., & Vincent, A. (1994). Mother' s little helpers: patterns of male care in mammals, (8), 294–297.

Wu, R., Song, Z., Tai, F., An, X., Yu, P., & Li, Y. (2013). The effect of alloparental experience and care on anxiety-like, social and parental behaviour in adult mandarin voles. *Animal Behaviour*, *85*(1), 61–69. https://doi.org/10.1016/j.anbehav.2012.10.007

Wu, R., Song, Z., Tai, F., Wang, L., Kong, L., & Wang, J. (2013). Post-weaning living with parents during juvenile period alters locomotor activity, social and parental behaviors in mandarin voles. *Behavioural Processes*, *98*, 78–84. https://doi.org/10.1016/j.beproc.2013.05.008

Xie, K., Ryan, D. P., Pearson, B. L., Henzel, K. S., Neff, F., Vidal, R. O., … Ehninger, D. (2018). Epigenetic alterations in longevity regulators, reduced life span, and exacerbated aging-related pathology in old father offspring mice. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), E2348–E2357. https://doi.org/10.1073/pnas.1707337115

Zhao, M., Garland, T., Chappell, M. A., Andrew, J. R., Saltzman, W., Garland Jr, T., … Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiology and Behavior*, *177*(April), 57–67. https://doi.org/10.1016/j.physbeh.2017.04.010

Ziegler, T. E. (2000). Hormones associated with non-maternal infant care: A review of mammalian and avian studies. *Folia Primatologica*, *71*(1–2), 6–21. https://doi.org/10.1159/000021726

Zöttl, M., Fischer, S., & Taborsky, M. (2013). Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour*, *85*(6), 1471–1478. https://doi.org/10.1016/j.anbehav.2013.03.045

Tables and Figures

Table 2.1. Reproductive measures (medians, first and third quartiles) of the first and second litters born to each focal family and results of Mann-Whitney U tests comparing old and young fathers.

		Old Fathers	**Young Fathers**	**Mann-Whitney**	
		N = 24	N = 25	U	P
First Litter	Number of pairs that produced first litter	18	18		
	Days from pairing until parturition	35.50 (34.75, 41.50)	36.50 (34.75, 37.75)	136.50	0.415
	Number of pups per litter	2 (2, 2)	2 (1, 3)	156.00	0.835
	Number of pups that survived past 1 week	2 (2, 2)	2 (1, 3)	125.00	0.220
	Proportion of male pups	0.50 (0.00, 1.00)	0.33 (0.00, 0.63)	56.00	0.393
Second Litter	Number of pairs that produced second litter	15	15		
	Interbirth interval (days)	34.00 (32.00, 36.00)	35.00 (33.00, 38.00)	84.00	0.228
	Number of pups per litter	3 (1, 3)	2 (1, 3)	104.50	0.726
	Number of pups that survived past 1 week	2 (0, 3)	2 (1, 3)	96.50	0.488

Table 2.2. Weekly body mass, lean and fat mass at weaning age (postpartum day [PPD] 28), and age of eye opening of offspring of old and young fathers (mean ± SE). Within each litter, all pups opened their eyes on the same day. Results of ANOVAs, nested by litter, comparing pups from old and young fathers, are shown both with and without the model corrected using the proportion of male pups as a covariate. p-values < 0.05 are in bold, and p-values > 0.05 and <0.1 are italicized.

			Body Mass (g)				PPD 28 Body Composition (g)		Age at Eye Opening (Days Postpartum)
	N (litters)	**N (pups)**	**PPD 7**	**PPD 14**	**PPD 21**	**PPD 28**	**Lean Mass**	**Fat Mass**	
Old Fathers	16	21	4.71 ± 0.17	8.75 ± 0.35	13.65 ± 0.70	18.11 ± 1.09	13.84 ± 2.58	1.82 ± 1.04	16.17 ± 0.51
Young Fathers	15	28	4.61 ± 0.17	7.71 ± 0.38	11.83 ± 0.70	15.00 ± 0.95	12.64 ± 2.93	1.53 ± 0.96	16.08 ± 0.49
p (Old Fathers vs Young Fathers)			0.704	*0.052*	*0.068*	**0.037**	0.318	0.448	0.898
F			0.146	3.968	3.470	4.591	1.175	0.818	0.017
p (Old Fathers vs Young Fathers, corrected for proportion of male pups)			0.929	0.141	0.169	*0.095*	0.139	0.298	0.900
			0.074	2.039	1.836	2.473	3.363	1.108	0.106

Table 2.3. Behavior (medians, first and third quartiles) of sexually naïve males in the parental-behavior test and results of Mann-Whitney U tests comparing old and young males. We defined parental behavior as grooming the pup, huddling over the pup, and/or nest building. For all latencies, animals that did not perform the behavior were assigned a latency of 3600s (the maximum duration of the test). P-values < 0.05 are in bold, and p-values 0.05 > and < 0.01 are italicized.

		Old Males	**Young Males**	**Mann-Whitney**	
		N = 24	N = 25	U	P
Parental Behavior	Latency to Initiate Parental Behavior (s)	833.78 (196.30, 3600.00)	656.45 (216.38, 3600.00)	314.00	0.767
	Duration of Huddling Pup (s)	151.72 (0.00, 1762.95)	0.00 (0.00, 1916.66)	297.00	0.949
	Duration of Grooming Pup (s)	629.24 (0.00, 1762.95)	5.15 (0.00, 828.21)	258.00	0.375
	Duration of Nest Building (s)	0.00 (0.00, 29.47)	0.00 (0.00, 98.38)	314.00	0.753
	Total Duration of Parental Behavior (s)	1284.00 (0.00, 3073.16)	5.15 (0.00, 3049.07)	300.00	1.000
Other Pup-directed Behavior	Latency to Sniff Pup (s)	36.28 (19.65, 235.09)	93.65 (53.65, 196.15)	395.50	*0.056*
	Latency to Contact Pup (s)	211.76 (39.82, 1581.77)	191.81 (88.53, 3600.00)	359.00	0.234
	Duration of Sniffing Pup (s)	63.50 (31.88, 183.63)	63.75 (38.50, 144.00)	291.50	0.865
	Duration of Contact with Pup (s)	21.63 (0.00, 78.27)	15.75 (0.00, 74.88)	293.50	0.895
Non-pup-directed Behavior	Duration of Locomotion (s)	185.63 (38.94, 508.34)	124.75 (31.87, 1027.87)	301.50	0.976
	Duration of Autogrooming (s)	172.50 (18.50, 303.08)	52.50 (20.78, 266.26)	254.00	0.356
	Duration of Resting (s)	26.25 (1.57, 143.69)	115.50 (58.37, 520.39)	422.00	**0.015**
	Total Duration of Non-pup-directed Behavior (s)	361.76 (205.12, 1688.05)	437.01 (182.62, 3142.05)	337.00	0.459

Table 2.4. Behaviors (medians, first and third quartiles) of fathers in an open-field arena for 10 minutes before (top) and 10 minutes after the introduction of an unfamiliar pup (bottom) and results of Mann-Whitney U tests comparing old and young fathers. For all behaviors, animals that did not perform the behavior were assigned a latency of 600 s, the maximum duration of the test. P-values <0.05 are in bold

		Old Fathers	**Young Fathers**	**Mann-Whitney**	
		N = 16	N = 15	U	P
Before Introduction of Pup	Duration in Center of Arena (s)	90.45 (49.75, 275.68)	202.83 (34.13, 567.33)	126.00	0.830
	Duration in Edges of Arena (s)	500.77 (321.85, 550.03)	441.37 (32.70, 582.50)	117.00	0.922
	Distance Traveled in Center (m)	17.44 (32.70, 582.50)	6.28 (0.86, 12.03)	59.00	**0.015**
	Distance Traveled in Periphery (m)	43.65 (14.51, 84.56)	9.31 (3.32, 33.53)	56.00	**0.011**
After Introduction of Pup	Latency to Approach Pup (s)	6.98 (3.99, 64.63)	24.03 (3.15, 199.78)	153.00	0.202
	Latency to Contact Pup (s)	7.88 (4.93, 65.70)	25.28 (6.65, 232.53)	157.00	0.151
	Duration of Locomotion (s)	305.03 (19.96, 400.42)	79.64 (22.00, 230.75)	140.00	0.264
	Duration in Contact with Pup (s)	212.38 (157.84, 471.29)	369.35 (112.75, 519.60)	133.00	0.626
	Duration of Resting (s)	8.75 (0.25, 41.36)	13.50 (0.00, 250.28)	91.00	0.446

Table 2.5. Results of Type III Ward tests examining the effects of postpartum week, paternal age, and their interaction on behavior of fathers in the home cage under undisturbed conditions. p-values < 0.05 are in bold, and p-values > 0.05 and < 0.1 are italicized.

		Grooming Pup		**Huddling over Pup**		**Physical Contact with Pup**		**General Locomotion**		**Eating**		**Physical Contact with Mate**	
Time of Day		χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
03:00 - 07:00	Postpartum Week	0.159	0.603	1.275	0.735	1.604	0.658	0.851	0.837	5.650	0.130	1.485	0.686
	Paternal Age	4.058	0.383	0.360	0.549	0.032	0.570	0.095	0.758	0.584	0.445	0.225	0.635
	Postpartum Week * Paternal Age	3.017	0.714	0.921	0.820	7.066	*0.070*	2.298	0.513	2.566	0.464	7.690	*0.053*
17:00 - 21:00	Postpartum Week	1.542	0.673	18.768	**< 0.001**	2.178	0.536	2.220	0.528	4.742	0.192	1.299	0.729
	Paternal Age	0.200	0.655	1.175	0.186	0.227	0.634	0.099	0.753	0.284	0.594	0.066	0.797
	Postpartum Week * Paternal Age	0.498	0.919	12.731	**0.005**	0.261	0.967	1.564	0.668	2.913	0.405	2.013	0.570

Table 2.6. Results of Type III Ward tests examining the effects of postpartum week, paternal age, and their interaction on behavior of mothers in the home cage under undisturbed conditions. p-values < 0.05 are in bold, and p-values > 0.05 and <0.1 are italicized.

		Grooming Pup		**Passive Nursing**		**General Locomotion**		**Eating**	
Time of Day		χ^2	p	χ^2	p	χ^2	p	χ^2	p
03:00 - 07:00	Postpartum Week	4.321	0.229	1.652	0.648	2.225	0.527	11.106	**0.011**
	Paternal Age	0.679	0.410	5.359	**0.021**	0.723	0.395	7.742	**0.005**
	Postpartum Week * Paternal Age	2.098	0.552	7.726	*0.052*	0.723	0.868	11.681	**0.009**
17:00 - 21:00	Postpartum Week	0.564	0.905	5.555	0.135	0.833	0.842	2.710	0.439
	Paternal Age	0.124	0.724	0.378	0.539	0.107	0.744	3.517	*0.061*
	Postpartum Week * Paternal Age	2.887	0.409	3.128	0.372	0.410	0.938	1.670	0.644

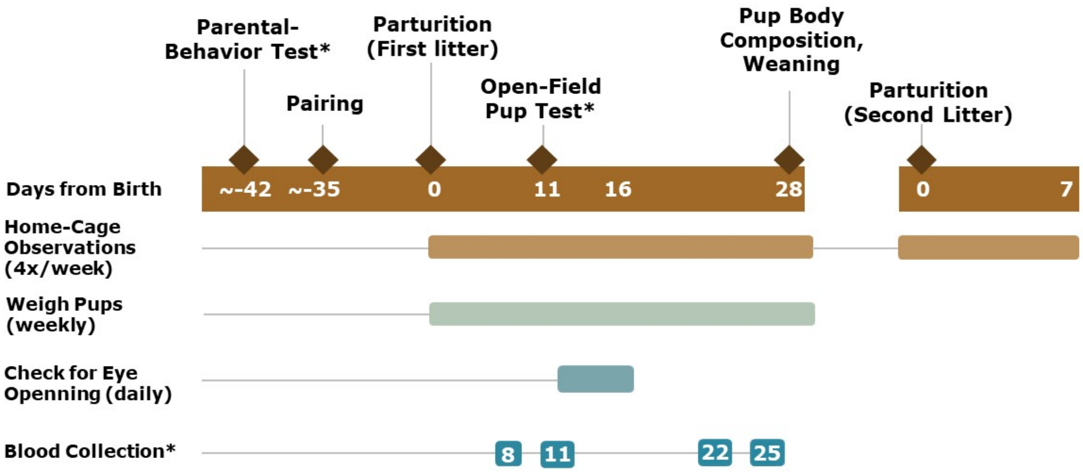

Figure 2.1. Timeline of experiment. Procedures and tests performed on fathers are indicated by *.

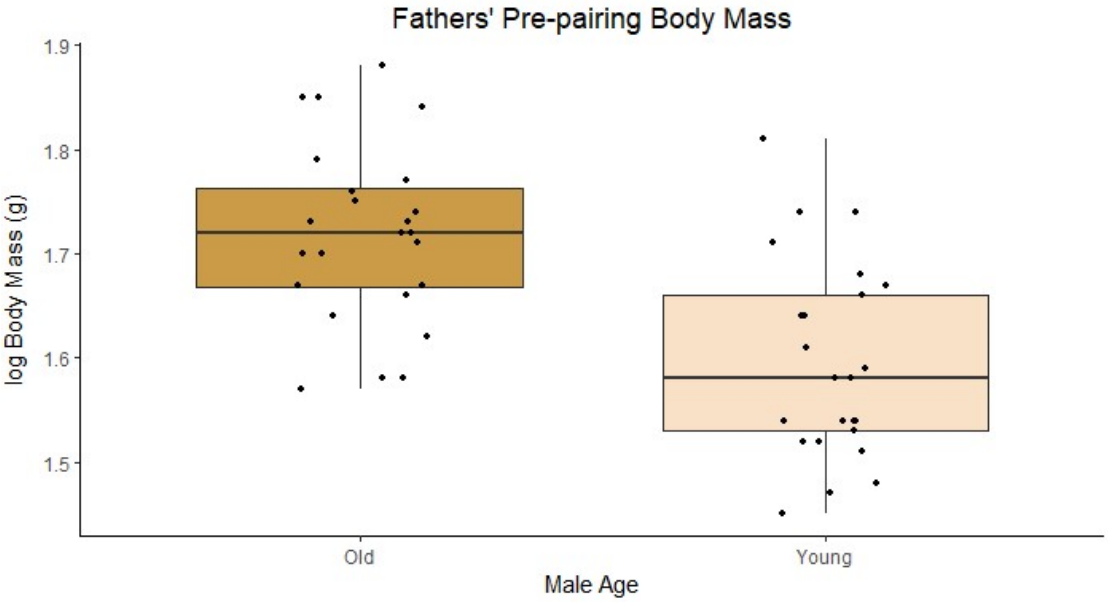

Figure 2.2. Log-transformed body masses of old (n = 24) and young (n = 25) reproductively naïve males one week prior to being paired with a female. Circles represent data from individual animals. Old males had significantly higher body mass than young males ($p < 0.001$).

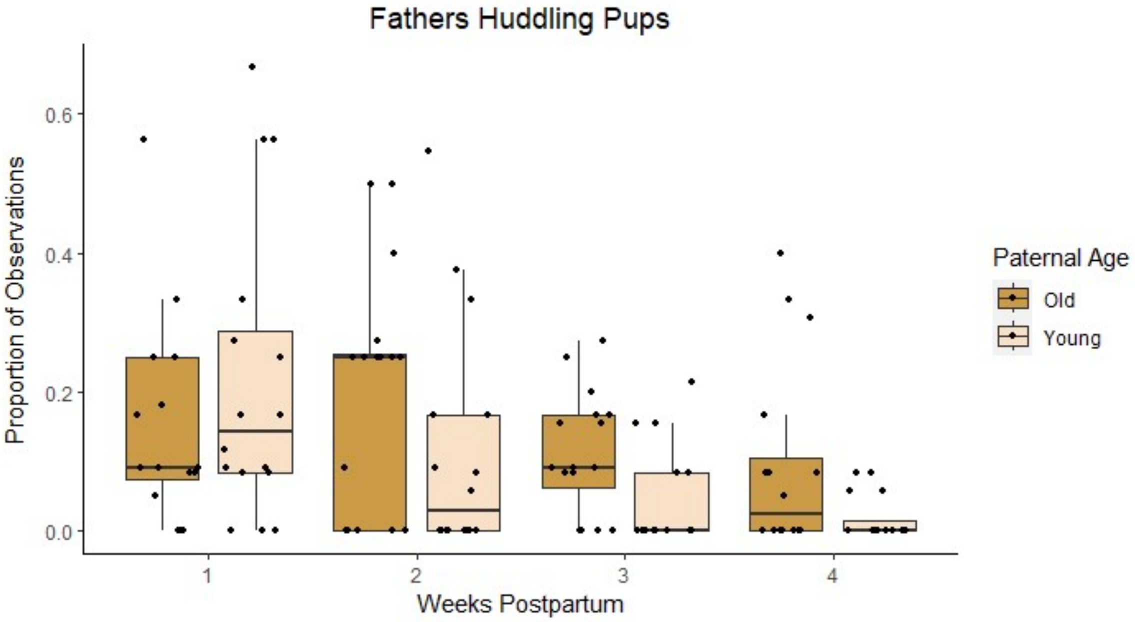

Figure 2.3. Proportions of scan samples in which old (n = 16) and young (n = 16) fathers huddled over their pups across the four-week pup-rearing period, during home-cage observations at 1700 – 2100 h (transition from lights on to lights off). Circles represent data from individual animals. During postpartum week 2, old fathers huddled over pups more frequently than old (p = 0.008) or young fathers during postpartum week 4 (p = 0.006). Young fathers huddled over pups more often during postpartum week 1 than during postpartum weeks 3 (p = 0.030) and 4 (p = 0.002).

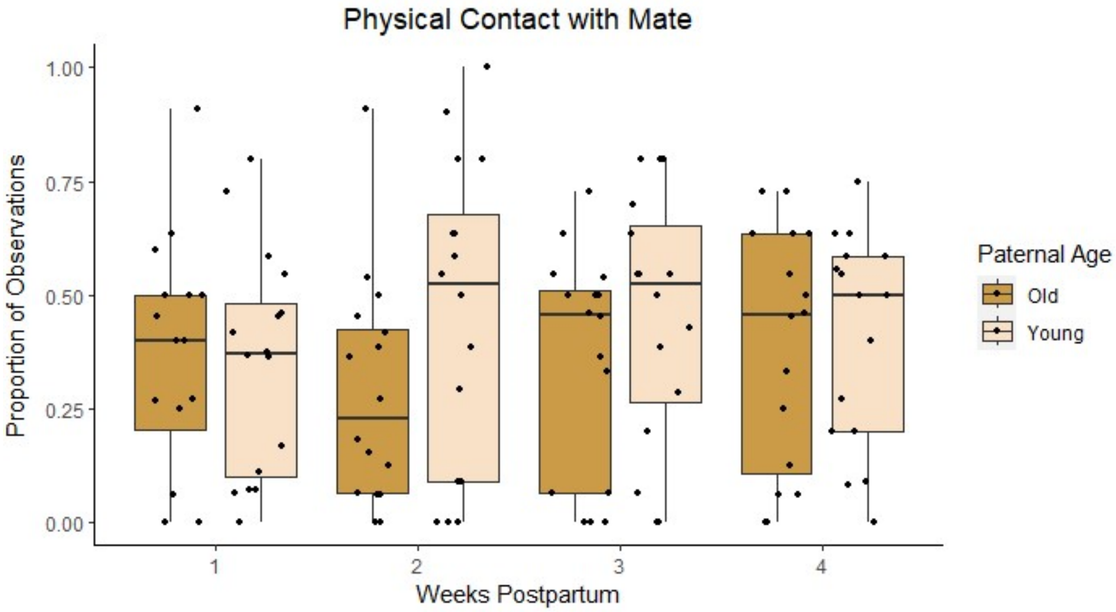

Figure 2.4. Proportions of scan samples in which fathers were in physical contact with their mate across the four-week pup-rearing period, in home-cage observations at 0300 – 0700 h (transition from lights off to lights on). Circles represent data from individual animals. Young fathers (n = 16) tended to be in contact with their mates more during postpartum week 2 than postpartum week 4 (p = 0.07).

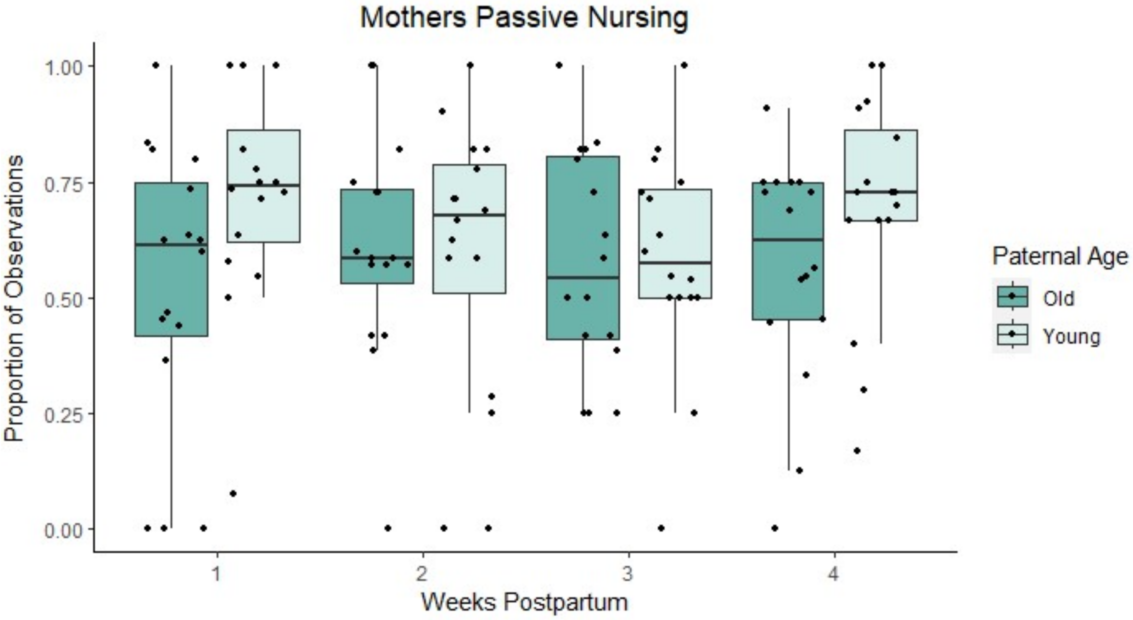

Figure 2.5. Proportions of scan samples in which mothers were passively nursing pups, during home-cage observations at 0300 – 0700 h. Circles represent data from individual animals. Mates of young fathers (n = 16) nursed pups more than mates of old fathers (n = 16, p = 0.021).

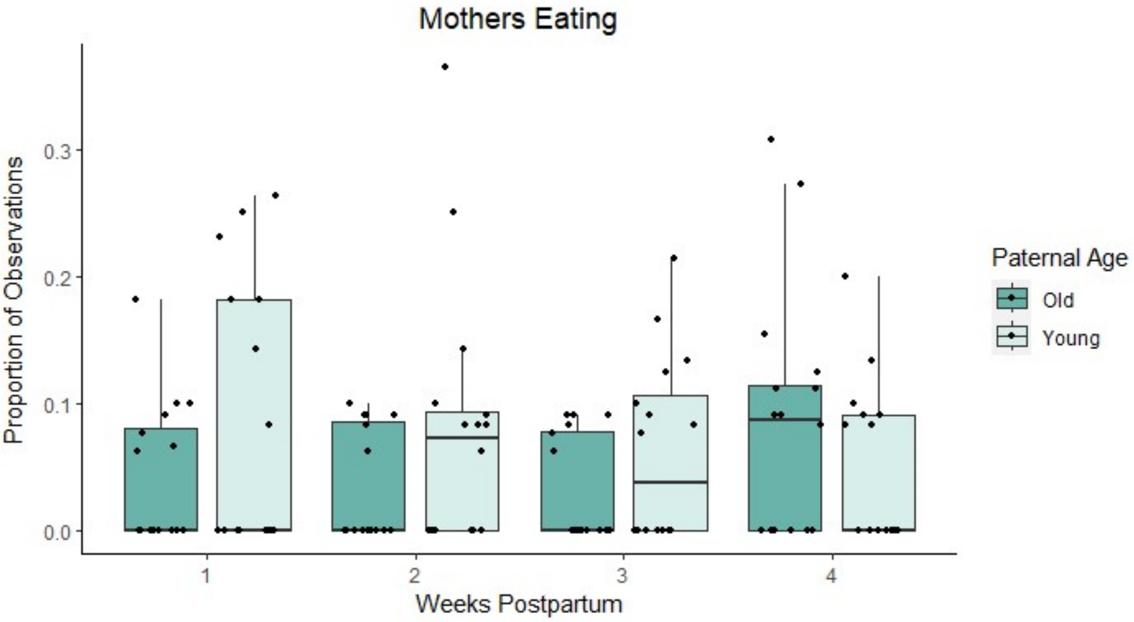

Figure 2.6. Proportions of scan samples in which mothers consumed food across the four-week pup-rearing period, during home-cage observations at 0300 – 0700 h. Circles represent data from individual animals. Mates of young fathers (n = 16) ate more during postpartum week 1 compared to mates of old fathers (n = 16) during postpartum weeks 2 (p = 0.020) and 3 (p = 0.018).

Chapter 3

Effects of Overlapping Litters on Family Dynamics and Offspring Development

Abstract

Early social environment can influence physiological and behavioral development, potentially affecting survival and reproductive success in adulthood. Much of the previous research in non-human animals has focused on interactions between parents and offspring or among littermates, but animals may also interact with siblings from previous or subsequent litters. Siblings may provide alloparental care or compete with each other, and parents may alter their behaviors in response. While many studies have identified long-term effects of alloparenting experience on older siblings, the effects of receiving alloparental care on younger siblings, competition between different-aged siblings, and parents' responses to offspring interactions have received little attention. In this study, we investigated the effects of overlapping litters on family dynamics and physiological and behavioral development in the California mouse (*Peromyscus californicus*). We found evidence that the presence of overlapping litters improves maternal condition but decreases body mass in older pups reared with younger siblings. Behaviorally, older offspring from overlapping litters may have heightened anxiety compared to older offspring living alone with their parents. Additionally, males reared with younger siblings are more parentally motivated than males that were not. Together, our results highlight potential costs and benefits of rearing overlapping litters in a biparental rodent.

1. Introduction

Early social environment has been shown to influence both physiological and behavioral development in animals, potentially affecting survival and reproductive success (Creel, Dantzer, Goymann, & Rubenstein, 2013; Hudson, Bautista, Reyes-Meza, Montor, & Rödel, 2011; Rödel, Von Holst, & Kraus, 2009). Much of this work has focused on interactions with parents or littermates (e.g. intrauterine position (Ryan & Vandenbergh, 2002), play behavior (Kross & Nelson, 2013), thermoregulatory huddling (González-Mariscal, Caba, Martínez-Gómez, Bautista, & Hudson, 2016), nursing competition (Hudson & Trillmich, 2008), but animals in some species may also interact with siblings from previous or succeeding litters. Overlapping litters, in which parents interact with offspring from consecutive bouts of reproduction concurrently, provide opportunities to investigate alloparental care, sibling competition, and parent-offspring conflict and their impact on offspring development.

In cooperative breeders, older siblings commonly provide alloparental care to younger siblings, which can have long-term consequences for the alloparent's anxiety-like behavior (Greenberg, van Westerhuyzen, Bales, & Trainor, 2012; Pillay & Rymer, 2015; Wu, Song, Tai, An, et al., 2013), exploration (Pillay & Rymer, 2015; Wu, Song, Tai, An, et al., 2013), ability to pair-bond with mates (Kenkel, Paredes, Yee, Bales, & Carter, 2012), and parental behavior towards their own offspring (Salo & French, 1989; Stone, Mathieu, Griffin, & Bales, 2010). In addition to behavioral effects, older siblings can benefit from providing alloparental through inclusive fitness. According to Hamilton's rule, animals should behave altruistically in proportion to the relatedness between themselves and those receiving the altruistic behavior and the costs and benefits of altruism (Hamilton, 1964). Although the genetic relatedness between an individual and both its full siblings and offspring is 0.5, providing care to offspring requires the appropriate resources to finding a mate and having young, while providing care to siblings does not involve this cost.

Younger siblings may benefit from receiving care in addition to that provided by parents (Wu, Song, Tai, An, et al., 2013). On the other hand, older and younger siblings may compete for resources (e.g., food, milk, thermoregulation, grooming) from their parents, potentially leading to detrimental effects on the younger animals, such as decreased growth and survival (Hudson & Trillmich, 2008). but the effects of interacting with older siblings in younger siblings have received little investigation.

Parents may discourage competition between offspring or mitigate the negative effects of sibling competition in order to maximize their own fitness (Trivers, 1974). In some biparental birds, parents divide caregiving between high- and low-quality offspring so that one type (e.g., low-quality) receives more care than the other (Royle, Smiseth, & Kölliker, 2012), although this was not examined in offspring from separate reproductive bouts. Parents can also attempt to encourage caregiving behavior in their older offspring. In common marmosets (*Callithrix jacchus*) and cotton-top tamarins (*Saguinus oedipus*) (Snowdon & Ziegler, 2007), parents have been observed to reward alloparents with grooming, while in meerkats (*Suricata suricatta)* (Clutton-Brock, 2004) and naked mole-rats (*Heterocephalus glaber*) (Reeve, 1992), parents behave aggressively towards non-breeders that perform low levels of alloparental care.

Mediating interactions among older and young offspring may lead to parent-offspring conflict. The optimal levels of parental care provided after weaning may differ between offspring and their parents (Trivers, 1974), and this conflict is likely to be exacerbated in the presence of younger offspring. Studies on birds and fish suggest that parents may alter their own levels of care in response to the presence of alloparents (Boheemen, Richardson, Burke, Komdeur, & Dugdale, 2019; Pike, Ashton, Morgan, & Ridley, 2019; Zöttl, Fischer, & Taborsky, 2013). In contrast, adjustments to parental behavior in the presence of alloparents have received little attention in mammals. Gestation and lactation are two energetically demanding processes performed in mammals; thus, alloparents can allow mothers to spend more time replenishing resources (Rosenbaum & Gettler, 2018). Therefore, mothers may reduce their levels of care when they have helpers (Clutton-brock, 2004; Crick, 1992). However, we have little empirical data on how mammalian fathers respond to the presence of helpers.

We investigated the effects of overlapping litters on family dynamics (parent-offspring and sibling-sibling interactions) and on physiological and behavioral development of offspring in California mice (*Peromyscus californicus*). Under field and laboratory conditions, this species is socially monogamous and biparental (Gubernick, 1988; Gubernick & Addington, 1994; Gubernick & Nordby, 1993), with both mothers and fathers providing care to litters of one to four pups (Gubernick & Alberts, 1987; Ribble, 1991). In the wild, weanlings disperse at 40 to 90 days of age, while gestation is approximately 30 days (Gubernick, 1988; Ribble, 1992a). Thus, given that postpartum estrus and conception occur in this species, it is possible for a new litter to be born before the previous litter disperses. Weanlings in this species have been observed interacting with their younger siblings and providing alloparental care to unfamiliar neonates, in an experimental context, until approximately 55 days of age (Gubernick & Laskin, 1994). However, alloparental care and sibling-sibling interactions have received very little attention compared to parental care in this species. We tested two hypotheses: H_1) older siblings will provide alloparental care due to benefits from inclusive fitness, and parents will reduce their own caregiving in response, and H_2) older and younger pups from overlapping litters will be of higher quality than pups raised in the absence of another litter. Alternatively, older and younger siblings could compete for resources from their parents, leading to detrimental effects on the physical and behavioral development of offspring from overlapping litters.

2. Methods

2.1.1. Animals -

We used California mice descended from animals purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC, USA). Mice were bred and housed at the University of California, Riverside (UCR) in polycarbonate cages (44 x 24 x 20 cm) under a 14:10 light:dark cycle (lights on at 23:00 h, lights off at 13:00 h) at approximately 22°C and 60-70% humidity. Aspen shavings and cotton were used for bedding and nesting material, respectively. Animals were provided food (Purina 5001 Rodent Chow, LabDiet, Richmond, IN, USA) and water ad lib. We checked animal health and births twice each day and changed cages weekly.

2.1.2. Experimental Design -

The experimental design is illustrated in Fig. 1. Parents in this study were weaned from their natal families at 27-31 days of age, housed in same-sex groups of 2-4 age-matched mice, and paired with an age-matched mate at 90-120 days of age. Pair mates were no more closely related than first cousins. Breeding pairs were housed in double cages, consisting of two standard cages connected by a curving, transparent plexiglass tunnel (5 cm diameter, 10 cm length; Fig. 2).

To evaluate the effects of overlapping litters on offspring behavior and development, we compared 1) first-born litters that were housed without (L1) or with (L1+) younger siblings, as well as 2) second-born litters that were housed without (L2) or with (L2+) older siblings. Thus, we formed three types of families: overlapping litters (OL families), first litter only (Litter 1 families), and second litter only (Litter 2 families). In the OL families, pups from the first litter remained with their parents during the rearing of the second litter, until the second litter was weaned at 27-31 days of age. OL families therefore contained first litters housed with younger siblings (L1+) as well as second litters reared with older siblings (L2+). To form Litter 1 families, we permanently removed the second litter on the day of birth (approximately 35 days after the birth of the first litter). Therefore, litters in Litter 1 families were raised without younger siblings present (i.e., L1). To form Litter 2 families, we permanently removed the first litter at postpartum day (PPD) 27, before the birth of the second litter; litters in Litter 2 families were therefore raised without older siblings present (i.e., L2). L1+ animals were housed with their families until the subsequent litter reached 27 days of age (approximately PPD 65); L1 animals were removed from their families at a matched time point. After weaning, focal offspring were housed in same-sex groups of 2-4 juveniles until paired with an opposite-sex mate at 90 days of age. In total, we formed 13 Litter 1 families, 12 Litter 2 families, and 13 OL families. Final numbers of pups in each condition are listed in Table 1.

2.2. Mass and Body Composition of Parents -

Starting one week after the mother's second parturition, we weighed both parents and measured their body composition (fat and lean mass) using MRI (EchoMRI-100, Echo Medical Systems, Houston, TX, USA; Zhao et al., 2017) once per week at 7-day intervals for the first four weeks after the birth of their second litter. This is a non-invasive method that lasts approximately 2 minutes and does not require anesthesia or sedation.

2.3. Pup Development -

Pups of all focal litters were weighed individually and had their body composition assessed using MRI once per week. We weighed and measured body composition starting at weaning age (i.e., 27 – 31 days of age) for L1+ and L1 juveniles and starting at 2 weeks of age for L2+ and L2 pups. L2+ and L2 pups were checked daily for eye opening starting at PPD 11 (Dudley, 1974).

2.4. Open-field Test on Offspring -

Early social environment, particularly the levels of care received, can influence the response to an acute stressor (Birnie, Taylor, Cavanaugh, & French, 2013; Francis, Champagne, Liu, & Meaney, 1999; Rödel, Meyer, Prager, Stefanski, & Hudson, 2010). Therefore, we tested all focal offspring in an open field, a common method of testing anxiety-like behavior in rodents (Ohl, 2003). At 42 days of age, approximately the age of dispersal in wild California mice (Ribble, 1992a), each focal animal was placed in an opaque, black acrylic open-field arena lined with white paper (1m x 1m x 0.4m) at 1400 – 1500 h for 10 minutes. The arena was illuminated from above by two lamps at 1400 lx (Perea-Rodriguez et al., 2018). A trained observer, blind to litter (i.e., L1+, L2+, L1, or L2) and family type (i.e., OL, Litter 1, or Litter 2), scored the duration of time the animal spent in the center 50% of the field and along the edges (i.e., outer 50%) of the field, the number of entries into and out of the center, and total distance traveled using TopScan video-tracking software (CleverSys, Reston, VA, USA, Perea-Rodriguez et al., 2018).

2.5. Parental-behavior Test on Offspring

After animals reached sexual maturity (approximately PPD 90), we tested parental responsiveness in a parental-behavior test between 1000 and 1100 h. California mice were removed from their home cage and placed in a clean standard cage with a thin layer of fresh bedding. After a 15-minute habituation period, an unrelated, unfamiliar 3- to 5-day-old pup was placed in the cage. The animals were video recorded for one hour unless the pup was injured, at which point the test was immediately terminated and the pup was euthanized. All animals were returned to their home cages after the test. Trained observers, blind to litter and family type, used BORIS (Friard & Gamba, 2016) to score the latency to approach the pup, latency to initiate parental behavior (i.e., grooming, huddling over, or carrying the pup), and durations of huddling over the pup, licking/grooming the pup, nestbuilding, general locomotion, autogrooming, and inactivity.

2.6. Statistical Analysis -

All analyses were performed in R (v.4.3.1, R Core Team, 2023). Body mass, fat mass, and lean mass of parents and offspring were transformed logarithmically prior to analysis. We compared body mass and composition (i.e., fat mass and lean mass) between parents from the three family types using repeated-measures ANCOVAs. Mothers and fathers were analyzed separately, and body mass was used as a covariate in analyses of fat and lean mass. Growth curves of focal offspring's total body, fat, and lean masses were compared between L1 and L1+ offspring and between L2 and L2+ offspring using linear mixed models. Condition (i.e., L1, L1+, L2, L2+), sex, and condition*sex were included as fixed effects, and individual animals were nested by litter using family ID as a random effect. For fat and lean mass, body mass was used as a covariate.

We compared behavior in the open-field test between L1+ and L1 litters and between L2+ and L2 litters using generalized linear mixed models (GLMM) using the *glmmtmb* package (Brooks et al., 2017). Condition and sex were included as fixed effects and family ID as a random effect to account for animals nested in the same litter. We used the *DHARMa* package in R (Hartig & Hartig, 2017) to check model fit, normality of residuals, and heterogeneity of variances and the *car* package (Fox & Weisberg, 2011) for posthoc analyses using pairwise comparisons. We used a critical P value of 0.05 (2-tailed) for all analyses.

Behavior during the parental-behavior test was analyzed using *glmmtmb* to compare L1+ to L1 litters and L2+ to L2 litters. Female and male focal offspring were analyzed separately, and we included litter type as a fixed effect and family ID as a random effect. For all behaviors, we first examined only animals that performed a given behavior to account for zeroes in the data; we then analyzed whether the number of animals that did and did not perform the behavior differed between litter types. We used gamma distributions to analyze the latencies to approach the pup, touch the pup, and initial parental behavior (licking and/or grooming). All other behaviors were divided by the maximum time for the parental behavior test (3600 s) in order to use a beta-family distribution for our models.

3. Results

3.1 Reproductive Outcomes

Total numbers of focal offspring produced by Litter 1, Litter 2, and OL families are listed in Table 1. Interbirth interval did not differ significantly between the three family types (Mean ± SE - L1: 39.0 ± 2.5 days, L2: 33.9. ± 0.8 days, OL: 36.2 ± 1.3 days, Kruskal-Wallis test, $\chi^2 = 4.89$, p = 0.09). Comparing second litters housed with (L2+) and without (L2) older siblings, we found no differences in litter size (T-test, t = -0.144, p = 0.91) or the ratio of males to females (Chi-Square test, $\chi^2 = 0.15$, p = 0.70).

3.2 Parental Body Mass and Composition

We recorded weekly body mass and body composition, starting from the birth of the second litter, from parents of 12 Litter 1 families, 8 Litter 2 families, and 13 OL families. We were unable to collect full sets of data from the other families due to technical issues (e.g., MRI machine malfunction). In mothers, body mass was significantly affected by family type ($F = 5.745$, $p < 0.01$, repeated-measures ANOVA) and week ($F = 4.408$, $p = 0.02$) but not the interaction between family type and week (Table 2). Post hoc comparisons revealed that OL mothers were significantly heavier than Litter 1 mothers ($p < 0.01$, pairwise-t-test with Bonferroni adjustment, Fig 3). We found no effects of family type or week on fathers' body mass (Fig 4). Fat and lean mass did not differ between family type for mothers or fathers when body mass was used as a covariate (Tables 2 and 3).

3.3 Pup Growth and Development

We recorded weekly body mass and body composition for 23 L1 (11 females and 12 males from a total of 13 litters) and 20 L1+ (7 females and 13 males from a total of 13 litters) focal offspring (Table 4), for the four weeks following the birth of their younger siblings. As expected, body mass increased progressively across weeks (Type III Wald χ^2 test, $\chi^2 = 74.9670$, $p < 0.01$). We also found a significant interaction between litter type and week (Type III Wald χ^2 test, $\chi^2 = 16.5270$, $p < 0.01$, Fig 5). Body mass of L1 offspring increased significantly between weeks 1 and 2 (T-test, $t = -4.790$, $p < 0.01$) and between weeks 2 and 3 (T-test, $t = -4.245$, $p < 0.01$). In contrast, body mass of L1+ offspring increased significantly only between weeks 1 and 2 (T-test, $t= -3.285$, $p = 0.03$) and marginally significantly between weeks 2 and 3 (T-test, $t = -2.975$, $p = 0.07$). No other significant effects of litter type, sex, week, or any interaction among them were observed for body mass of older offspring (Table 4).

We found a marginally significant effect of sex on fat mass in L1 and L1+ offspring: Female offspring, regardless of litter type and week, tended to have higher fat mass than male offspring (T-test, $t = 1.864$ $p = 0.07$). Conversely, male offspring tended to have higher lean mass than female offspring (T-test, $t = -1.81$. $p = 0.07$). Offspring lean mass also increased weekly (Type III Wald χ^2 test, $\chi^2 = 6.41$, $p < 0.01$). No other effects of sex, condition, week, or interactions among them were found on fat or lean mass of older offspring (Table 4).

Weekly mass and body composition were collected from 19 L2 pups from 8 litters and 32 L2+ pups from 12 litters. Body mass of all younger offspring increased progressively across weeks (Type III Wald χ^2 test, $\chi^2 = 264.25$, $p < 0.01$). However, body mass was not significantly influenced by litter type or the interaction between litter type and week. Fat mass, in contrast, was significantly affected by an interaction between week and litter type (Type III Wald χ^2 test, $\chi^2 = 3.50$, $p < 0.03$). L2+ offspring showed a significant gain in fat mass between Weeks 2 and 3 ($t = 2.96$, $p = 0.04$), whereas no other pairwise comparisons between weeks were significant for either L2 or L2+ offspring. We did not find any significant effects of litter type or week on lean mass of younger offspring (Table 5). L2 and L2+ offspring also did not significantly differ in the age at which their eyes first opened (T-test, $t = 1.86$, $p = 0.08$).

3.4 Open-field Test

All focal offspring were tested in the open-field test at approximately 42 days of age. L1 offspring traveled greater distances in the center of the open field than L1+ offspring (Type III Wald χ^2 test, $\chi^2 = 3.84$, $p = 0.05$, Fig. 6). In addition, males traveled greater distances in the outer portion and in total than females, regardless of family type (Type III Wald χ^2 test, $\chi^2 = 4.54$, $p = 0.03$). No other behaviors differed significantly between males and females or between L1 and L1+ offspring, and none differed between L2 and L2+ offspring (Table 6 and 7).

3.5. Parental-behavior Test

At approximately 90 days of age, we tested each focal animal with an unfamiliar pup. Of the 37 female offspring that were tested, 11 behaved parentally (i.e., licked or huddled) toward the pup, 5 attacked the pup, and 21 behaved neutrally (i.e., neither behaved parentally nor attacked) toward the pup (Table XXX). Of the 35 male offspring tested, 22 behaved parentally, 4 attacked the pup, and 9 behaved neutrally (Table XXX). The proportion of offspring that behaved parentally, aggressively, or neutrally did not differ among litter types in either sex (χ^2 test, $p > 0.05$). Additionally, the number of animals that did and did not perform a given behavior (i.e. XXX) did not differ among litter type in either sex (χ^2 tests, $p > 0.05$)

We found no significant differences in behavior between L1+ and L1 females or L2+ and L2 females (Table 8). In males, we found a significant effect of litter type on the latency to approach the pup (Type III Wald χ^2 test, $\chi^2 = 4.88$, $p = 0.03$) and touch (Type III Wald χ^2 test, $\chi^2 = 4.42$, $p = 0.04$). Males that had been reared with younger siblings (i.e., L1+ males) approached (z-test, $z = -2.21$, $p = 0.03$) and touched (z-test, $z = -2.10$, $p = 0.03$) the pup more quickly than males reared without younger siblings (i.e., L1 males). We also observed a significant effect of litter type on bedding manipulation (Type III Wald χ^2 test, $\chi^2 = 10.175$, $p < 0.01$). Litter type had no other significant effects on behaviors of L1 versus L1+ males (Table 9). Numbers of males in younger litters for which parental-behavior test data were available were insufficient for statistical analysis.

4. Discussion

In this study, we explored the effects of overlapping litters on within-family dynamics and behavioral and physiological development of offspring. To do this, we tested the hypotheses that, in California mice, 1) older siblings will provide alloparental care and 2) older and younger siblings will both benefit from being raised together. Overall, we found some evidence to suggest the presence of alloparental care in this species and some positive and negative effects of overlapping litters on offspring growth and behavior as adults.

4.1. Effects of Overlapping Litters on Parents

We found no significant differences between fathers from different Litter 1, Litter 2, and Overlapping Litters (OL) families. However, OL mothers were heavier than Litter 1 mothers, suggesting an effect of presence of younger offspring. Possibly, L1+ offspring provided alloparental care to their younger siblings in OL families, allowing their mothers more time and energy to devote to foraging and other self-maintenance behaviors (Crick, 1992). On the other hand, the difference in body mass between OL and Litter 1 mothers might be explained by L1 offspring continuing to solicit and receive resources from their mothers. California mouse pups have tenacious nipple attachment (Gilbert, 1995), and we have observed ~28-31-day-old offspring still latched on to their mothers' teats despite being able to consume solid food (personal observations). However, it is important to note that suckling behavior does not necessarily mean that offspring are receiving milk (Cameron, 1998).

4.2. Effects of Overlapping Litters on Offspring Development

The growth of older offspring was impacted by the presence of younger siblings in that L1 offspring had a statistically significant increase in body mass between weeks 2 and 3, but L1+ offspring only had a marginally significant increase. Given that neither L1 nor L1+ offspring had a significant increase in body mass between weeks 3 and 4, our results may indicate that growth plateaus earlier in L1+ offspring than L1 offspring. If L1+ offspring provided alloparental care to their younger siblings, then they possibly spent less time foraging than L1 offspring (Heinsohn & Legge, 1999; Stead, Mucha, & Bădescu, 2019). Another reason for this result could be that L1+ offspring experienced conflicts with their young siblings in addition to littermates while L1 offspring did not. In Mongolian gerbils (Scheibler, Weinandy, & Gattermann, 2005) and cotton-top tamarins (Snowdon & Pickhard, 1999), both cooperatively breeding species, intra-family aggression increases after family size reaches a threshold number of individuals, which is suggested to facilitate dispersal. Alternatively, L1 offspring might have obtained more milk from their mothers than L1+ offspring, consistent with our finding that Litter 1 mothers weighed less than OL mothers. In some species, delaying dispersal can allow offspring to retain access to food, protection by parents, and other resources at the natal site (reviewed in Komdeur & Ekman, 2010). Postponing dispersal may, therefore, improve offspring survival until they can find suitable territories of their own, incentivizing parents to tolerate the presence of older offspring, regardless of alloparental care (reviewed in Komdeur & Ekman, 2010).

Few differences were found in body, fat, and lean mass between younger offspring reared with and without older siblings, suggesting that the presence of older offspring had little effect on the physical development of pups. However, this study was conducted using a captive colony in which animals were given food and water ad lib, temperature and humidity were constant, and no predators were present. It is possible that effects of the presence of older siblings would be apparent under natural conditions.

4.3 Effects of Overlapping Litters on Behavior in Adulthood

We observed that L1 offspring traveled greater distances in the center of the open field than L1+ offspring. The open-field test is commonly used to test anxiety-like behavior in rodents (Archer, 1975); thus, L1 offspring displayed less anxiety-like behavior than L1+ offspring. The age at which focal offspring were tested in the open field is approximately that at which wild offspring begin to disperse (Ribble, 1992). Similar increases in anxiety-like behavior and decreases in exploratory behavior in older offspring, compared to younger siblings, have been observed in other rodents (Greenberg et al., 2012; Stone et al., 2010; Wu, Song, Tai, Wang, et al., 2013). Wu et al. suggested that due to delayed dispersal, alloparents may have had fewer opportunities or less necessity to explore in order to locate resources (e.g., food, suitable habitat, mates).

Interestingly, male offspring, regardless of litter type, traveled greater distances in the periphery of the open field than female offspring. This contradicts findings of previous studies in California mice that found no sex differences in exploratory and/or anxiety-like behavior (Jašarević, Williams, Roberts, Geary, & Rosenfeld, 2012; Nguyen et al., 2020). Additionally, field studies in California mice have found that males tend to be philopatric to their natal site and disperse shorter distances than females which may suggest that they should display less exploratory behavior (Ribble, 1992a).

During the parental-behavior test, L1 males were slower to approach and touch unfamiliar pups compared to L1+ males. This result suggests that male offspring that interacted with younger siblings are more willing to interact with pups than male offspring that did not. In contrast, female focal offspring of different litter types did not differ in pup-directed behavior. Our lab previously found that sexually naïve males displayed more parental behaviors than sexually naïve females (Nguyen et al., 2020). Possibly, the effects of overlapping litters on response to unrelated, unfamiliar pups may be stronger in male than female offspring.

We found no other differences between L1 and L1+ or between L2 and L2+ animals in pup-directed behavior during the parental-behavior test; therefore, we do not have conclusive evidence that the presence of younger siblings increases parental responsiveness in California mice. L1 males were observed manipulating bedding material for longer durations than L1+ males. Although the manipulation of bedding material could be a parental behavior associated with building a nest for young, this behavior was performed by animals both that did and did not initiate other caregiving behaviors (i.e., huddling and grooming of pups). We did not, however, differentiate between animals that manipulated bedding while near and away from the pup.

Overall, rearing overlapping litters improved maternal condition but had mixed effects on offspring. Although L1+ males, which were housed with younger siblings during the juvenile period, showed greater interest in pups than L1 males, L1+ offspring had decreased growth rate and displayed more anxiety-like behavior in the open field compared to L1 offspring. Unexpectedly, we found no differences between L2 and L2+ offspring. Our results contribute to the understanding of an unexplored aspect of California mouse family dynamics and warrant further investigation into the presence of alloparental care and its effects on parents and offspring.

References

Ahern, T. H., Hammock, E. A. D., & Young, L. J. (2010). Parental Division of Labor , Coordination , and the Effects of Family Structure on Parenting in Monogamous Prairie Voles (Microtus ochrogaster). https://doi.org/10.1002/dev.20498

Angelier, F., Shaffer, S. A., Weimerskirch, H., & Chastel, O. (2006). Effect of age, breeding experience and senescence on corticosterone and prolactin levels in a long-lived seabird: The wandering albatross. *General and Comparative Endocrinology*, *149*(1), 1–9. https://doi.org/10.1016/j.ygcen.2006.04.006

Angell, C. S., Janacek, R., & Rundle, H. D. (2022). Maternal and Paternal Age Effects on Male Antler Flies: A Field Experiment. *American Naturalist*, *199*(3), 436–442. https://doi.org/10.1086/718236

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology*, *14*(4), 451–479.

Auroux, M. (1983). Decrease of learning capacity in offspring with increasing paternal age in the rat. *Teratology*, *27*(2), 141–148. https://doi.org/10.1002/tera.1420270202

Bales, K. L., & Saltzman, W. (2016). Fathering in rodents: Neurobiological substrates and consequences for offspring. *Hormones and Behavior*, *77*, 249–259. https://doi.org/10.1016/j.yhbeh.2015.05.021

Benowitz, K. M., Head, M. L., Williams, C. A., Moore, A. J., & Royle, N. J. (2013). Male age mediates reproductive investment and response to paternity assurance. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1764), 20131124. https://doi.org/10.1098/rspb.2013.1124

Bester-Meredith, J. K., Burns, J. N., Conley, M. F., Mammarella, G. E., & Ng, N. D. (2017). Peromyscus as a model system for understanding the regulation of maternal behavior. *Seminars in Cell and Developmental Biology*, *61*, 99–106. https://doi.org/10.1016/j.semcdb.2016.07.001

Birnie, A. K., Taylor, J. H., Cavanaugh, J., & French, J. A. (2013). Quality of maternal and paternal care predicts later stress reactivity in the cooperatively-breeding marmoset (Callithrix geoffroyi). *Psychoneuroendocrinology*, *38*(12), 3003–3014. https://doi.org/10.1016/j.psyneuen.2013.08.011

Boheemen, L. A. Van, Richardson, D. S., Burke, T., Komdeur, J., & Dugdale, H. L. (2019). Compensatory and additive helper effects in the cooperatively breeding Seychelles warbler (Acrocephalus sechellensis), (October 2018), 2986–2995. https://doi.org/10.1002/ece3.4982

Bolton, J. L., Molet, J., Ivy, A., & Baram, T. Z. (2017). New insights into early-life stress and behavioral outcomes. *Current Opinion in Behavioral Sciences*, *14*, 133–139. https://doi.org/10.1016/j.cobeha.2016.12.012

Braun, K., & Champagne, F. A. (2014). Paternal influences on offspring development: Behavioural and epigenetic pathways. *Journal of Neuroendocrinology*, *26*(10), 697–706. https://doi.org/10.1111/jne.12174

Bridges, R. S. (2016). Long-term alterations in neural and endocrine processes induced by motherhood in mammals. *Hormones and Behavior*, *77*, 193–203. https://doi.org/10.1016/j.yhbeh.2015.09.001

Brooks, M. E., Kristensen, K., Benthem, K. J. van, Magnusson, A., Berg, C. W., Nielsen, A., … Bolker, B. M. (2017). Modeling zero-inflated count data with glmmTMB. *BioRxiv*, 132753. Retrieved from https://www.biorxiv.org/content/10.1101/132753v1%0Ahttps://www.biorxiv.org/content/10.1101/132753v1.abstract

Burley, N. (1988). The differential-allocation hypothesis: an experimental test. *American Naturalist*, *132*(5), 611–628. https://doi.org/10.1086/284877

Caballero-Campo, P., Lin, W., Simbulan, R., Liu, X., Feuer, S., Donjacour, A., & Rinaudo, P. F. (2018). Advanced Paternal Age Affects Sperm Count and Anogenital Distance in Mouse Offspring. *Reproductive Sciences*, *25*(4), 515–522. https://doi.org/10.1177/1933719118759441

Caldji, C., Diorio, J., & Meaney, M. J. (2000). Variations in maternal care in infancy regulate the development of stress reactivity. *Biological Psychiatry*, *48*(12), 1164–1174. https://doi.org/10.1016/S0006-3223(00)01084-2

Caldwell, H. K. (2018). Oxytocin and sex differences in behavior. *Current Opinion in Behavioral Sciences*, *23*, 13–20. https://doi.org/10.1016/j.cobeha.2018.02.002

Cameron, E. Z. (1998). Is suckling behavior a useful predictor of milk intake? A review. *Animal Behaviour*, *56*(3), 521–532. https://doi.org/10.1006/anbe.1998.0793

Cameron, E. Z., Linklater, W. L., Stafford, K. J., Minot, E. O. 0, Minot, E. O. 0, Cameron, E. Z., … Minot, E. O. 0. (2000). Aging and Improving Reproductive Success in Horses: Declining Residual Reproductive Value or Just Older and Wiser? *Source: Behavioral Ecology and Sociobiology Behav Ecol Sociobiol*, *47*(47), 243–249. Retrieved from http://www.jstor.org/stable/4601737

Cantoni, D., Glaizot, O., & Brown, R. E. (1999). Effects of sex composition of the litter on anogenital distance in California mice (Peromyscus californicus). *Canadian Journal of Zoology*, *77*(1), 124–131. https://doi.org/10.1139/cjz-77-1-124

Carrageta, D. F., Guerra-carvalho, B., Angélica, M., Marc, S., Oliveira, P. F., & Alves, M. G. (2022). Animal models of male reproductive ageing to study testosterone production and spermatogenesis, 1341–1360.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, *29*(3), 386–397. https://doi.org/10.1016/j.yfrne.2008.03.003

Cholewa, M., Jankowiak, Ł., Szenejko, M., Dybus, A., Śmietana, P., & Wysocki, D. (2021). The effects of parental age difference on the offspring sex and fitness of European blackbirds. *PeerJ*, *9*, 1–20. https://doi.org/10.7717/peerj.10858

Clark, M. M., Moghaddas, M., & Galef, B. G. (2002). Age at first mating affects parental effort and fecundity of female Mongolian gerbils. *Animal Behaviour*, *63*(6), 1129–1134. https://doi.org/10.1006/anbe.2002.3019

Clutton-brock, T. H. (2004). Cooperative breeding in mammals.

Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, New Jersey, USA: Princeton University Press.

Creel, S., Dantzer, B., Goymann, W., & Rubenstein, D. R. (2013). The ecology of stress: Effects of the social environment. *Functional Ecology*, *27*(1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x

Crick, H. Q. P. (1992). Load-lightening in cooperatively breeding birds and the cost of reproduction. *Ibis*, *134*(1), 56–61. https://doi.org/10.1111/j.1474-919X.1992.tb07230.x

Dudley, D. (1974). Contributions of paternal care to the growth and development of the young in Peromyscus californicus. *Behavioral Biology*, *11*(2), 155–166. https://doi.org/10.1016/S0091-6773(74)90305-8

English, S. (2009). Individual variation in cooperative behaviour in meerkats, (October).

Ericsson, G., Wallin, K., Ball, J. P., & Broberg, M. (2001). Age-related reproductive effort and senescence in free-ranging moose, Alces alces. *Ecology*, *82*(6), 1613–1620. https://doi.org/10.1890/0012-9658(2001)082[1613:ARREAS]2.0.CO;2

Fay, R., Barbraud, C., Delord, K., & Weimerskirch, H. (2016). Paternal but not maternal age influences early-life performance of offspring in a long-lived seabird. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1828). https://doi.org/10.1098/rspb.2015.2318

Fisher, R. A. (1930). *The genetical theory of natural selection. The genetical theory of natural selection.* Oxford, England: Clarendon Press. https://doi.org/10.5962/bhl.title.27468

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Sage publications.

Francis, D. D., Champagne, F. A., Liu, D., & Meaney, M. J. (1999). Maternal care, gene expression, and the development of individual differences in stress reactivity. *Annals of the New York Academy of Sciences*, *896*, 66–84. https://doi.org/10.1111/j.1749-6632.1999.tb08106.x

Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

García-Palomares, S., Navarro, S., Pertusa, J. F., Hermenegildo, C., García-Pérez, M. A., Rausell, F., … Tarín, J. J. (2009). Delayed Fatherhood in Mice Decreases Reproductive Fitness and Longevity of Offspring. *Biology of Reproduction*, *80*(2), 343–349. https://doi.org/10.1095/biolreprod.108.073395

García-Palomares, S., Pertusa, J. F., Miñarro, J., García-Pérez, M. A., Hermenegildo, C., Rausell, F., … Tarín, J. J. (2009). Long-Term Effects of Delayed Fatherhood in Mice on Postnatal Development and Behavioral Traits of Offspring. *Biology of Reproduction*, *80*(2), 337–342. https://doi.org/10.1095/biolreprod.108.072066

Gilbert, A. N. (1995). Tenacious nipple attachment in rodents: the sibling competition hypothesis. *Animal Behaviour*, *50*(4), 881–891. https://doi.org/10.1016/0003-3472(95)80091-3

González-Mariscal, G., Caba, M., Martínez-Gómez, M., Bautista, A., & Hudson, R. (2016). Mothers and offspring: The rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. *Hormones and Behavior*, *77*, 30–41. https://doi.org/10.1016/j.yhbeh.2015.05.011

Greenberg, G. D., van Westerhuyzen, J. A., Bales, K. L., & Trainor, B. C. (2012). Is it all in the family? The effects of early social structure on neural-behavioral systems of prairie voles (Microtus ochrogaster). *Neuroscience*, *216*, 46–56. https://doi.org/10.1016/j.neuroscience.2012.04.063

Gromov, V. S. (2009). Interactions of partners in family pairs, care of the offspring, and the role of tactile stimulation in formation of parental behavior of the Mongolian gerbil (Meriones unguiculatus) under laboratory conditions. *Biology Bulletin*, *36*(5), 479–488. https://doi.org/10.1134/s1062359009050082

Gubernick, D. J. (1988). Reproduction in the California Mouse, Peromyscus californicus. *Journal of Mammalogy*, *69*(4), 857–860. https://doi.org/10.2307/1381649

Gubernick, D. J., & Addington, R. L. (1994). The stability of female social and mating preferences in the monogamous California mouse, peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1079

Gubernick, D. J., & Alberts, J. R. (1987). The biparental care system of the California mouse, Peromyscus californicus.. *Journal of Comparative Psychology*, *101*(2), 169–177. https://doi.org/10.1037/0735-7036.101.2.169

Gubernick, D. J., & Laskin, B. (1994). Mechanisms influencing sibling care in the monogamous biparental California mouse, Peromyscus californicus. *Animal Behaviour*. https://doi.org/10.1006/anbe.1994.1356

Gubernick, D. J., & Nordby, J. C. (1992). Parental influences on female puberty in the monogamous California mouse, Peromyscus californicus. *Animal Behaviour*, *44*(PART 2), 259–267. https://doi.org/10.1016/0003-3472(92)90032-5

Gubernick, D. J., & Nordby, J. C. (1993). Mechanisms of sexual fidelity in the monogamous California mouse, Peromyscus californicus. *Behavioral Ecology and Sociobiology*, *32*(3), 211–219. https://doi.org/10.1007/BF00173779

Gubernick, D. J., & Teferi, T. (2000). Adaptive significance of male parental care in a monogamous mammal. *The Royal Society*, *267*(1439), 147–150. https://doi.org/10.1098/rspb.2000.0979

Hamilton, W. D. (1964). The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, *7*(1), 1–16. https://doi.org/10.1016/0022-5193(64)90038-4

Hardy, M. P., & Schlegel, P. N. (2004). Testosterone Production in the Aging Male: Where Does the Slowdown Occur? *Endocrinology*, *145*(10), 4439–4440. https://doi.org/10.1210/en.2004-0888

Harper, L. V. (1981). Offspring effects upon parents. In *Parental care in mammals* (pp. 117–177). Springer.

Harris, B. N., & Saltzman, W. (2013). Effects of aging on hypothalamic-pituitary-adrenal (HPA) axis activity and reactivity in virgin male and female California mice (Peromyscus californicus). *General and Comparative Endocrinology*, *186*, 41–49. https://doi.org/10.1016/j.ygcen.2013.02.010

Harrison, F., Barta, Z., Cuthill, I., & Székely, T. (2009). How is sexual conflict over parental care resolved? A meta-analysis. *Journal of Evolutionary Biology*, *22*(9), 1800–1812. https://doi.org/10.1111/j.1420-9101.2009.01792.x

Hartig, F., & Hartig, M. F. (2017). Package 'DHARMa.' *R Package*.

Havighorst, A., Crossland, J., & Kiaris, H. (2017). Peromyscus as a model of human disease. *Seminars in Cell and Developmental Biology*, *61*, 150–155. https://doi.org/10.1016/j.semcdb.2016.06.020

Heinsohn, R., & Legge, S. (1999). The cost of helping. *Trends in Ecology and Evolution*, *14*(2), 53–57. https://doi.org/10.1016/S0169-5347(98)01545-6

Houslay, T. M., Kitchener, P. A., & Royle, N. J. (2020). Are older parents less flexible? Testing age-dependent plasticity in Nicrophorus vespilloides burying beetles. *Animal Behaviour*, *162*, 79–86. https://doi.org/https://doi.org/10.1016/j.anbehav.2020.01.012

Houston, A. I., Székely, T., & McNamara, J. M. (2005). Conflict between parents over care. *Trends in Ecology and Evolution*, *20*(1), 33–38. https://doi.org/10.1016/j.tree.2004.10.008

Hudson, R., Bautista, A., Reyes-Meza, V., Montor, J. M., & Rödel, H. G. (2011). The effect of siblings on early development: A potential contributor to personality differences in mammals. *Developmental Psychobiology*, *53*(6), 564–574. https://doi.org/10.1002/dev.20535

Hudson, R., & Trillmich, F. (2008). Sibling competition and cooperation in mammals: Challenges, developments and prospects. *Behavioral Ecology and Sociobiology*, *62*(3), 299–307. https://doi.org/10.1007/s00265-007-0417-z

Jašarević, E., Williams, S. A., Roberts, R. M., Geary, D. C., & Rosenfeld, C. S. (2012). Spatial navigation strategies in Peromyscus: A comparative study. *Animal Behaviour*, *84*(5), 1141–1149. https://doi.org/10.1016/j.anbehav.2012.08.015

Jia, R., Tai, F., An, S., & Zhang, X. (2011). Neonatal paternal deprivation or early deprivation reduces adult parental behavior and central estrogen receptor α expression in mandarin voles (Microtus mandarinus). *Behavioural Brain Research*, *224*(2), 279–289. https://doi.org/10.1016/j.bbr.2011.05.042

Johnson, S. L., & Gemmell, N. J. (2012). Are old males still good males and can females tell the difference?: Do hidden advantages of mating with old males off-set costs related to fertility, or are we missing something else. *BioEssays*, *34*(7), 609–619. https://doi.org/10.1002/bies.201100157

Johnstone, R. A., & Hinde, C. A. (2006). Negotiation over offspring care — how should parents respond to each other' s efforts ?, (July). https://doi.org/10.1093/beheco/arl009

Jurkevich, A., Grossmann, R., Balthazart, J., & Viglietti-Panzica, C. (2001). Gender-related changes in the avian vasotocin system during ontogeny. *Microscopy Research and Technique*, *55*(1), 27–36. https://doi.org/10.1002/jemt.1153

Kenkel, W. M., Paredes, J., Yee, J. R., Bales, K. L., & Carter, C. S. (2012). Neuroendocrine and Behavioural Responses to Exposure to an Infant in Male Prairie Voles Neuroendocrinology, (12), 874–886. https://doi.org/10.1111/j.1365-2826.2012.02301.x

Komdeur, J., & Ekman, J. (2010). Adaptations and constraints in the evolution of delayed dispersal: implications for cooperation. *Social Behaviour: Genes, Ecology and Evolution. Cambridge Univ. Press, Cambridge*, 306–327.

Kross, S. M., & Nelson, X. J. (2013). Factors influencing the behavioural development of juvenile New Zealand Falcons (Falco novaeseelandiae). *Emu*, *113*(1), 84–87. https://doi.org/10.1071/MU12020

Lecomte, V. J., Sorci, G., Cornet, S., Jaeger, A., Faivre, B., Arnoux, E., … Chastel, O. (2010). Patterns of aging in the long-lived wandering albatross. *Proceedings of the National Academy of Sciences*, *107*(14), 6370–6375. https://doi.org/10.1073/pnas.0911181107

Lemaître, J. F., & Gaillard, J. M. (2017). Reproductive senescence: new perspectives in the wild. *Biological Reviews*, *92*(4), 2182–2199. https://doi.org/10.1111/brv.12328

Lonstein, J. S., & De Vries, G. J. (2000). Sex differences in the parental behavior of rodents. *Neuroscience and Biobehavioral Reviews*, *24*(6), 669–686. https://doi.org/10.1016/S0149-7634(00)00036-1

Mashoodh, R., Franks, B., Curley, J. P., & Champagne, F. A. (2012). Paternal social enrichment effects on maternal behavior and offspring growth. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(SUPPL.2), 17232–17238. https://doi.org/10.1073/pnas.1121083109

McCabe, T. T., & Blanchard, B. D. (1950). Three species of Peromyscus.

Merritt, J. F. (1978). Peromyscus californicus. *Mammalian Species*, (85), 1. https://doi.org/10.2307/3503909

Miyamoto, M., Kiyota, Y., Nishiyama, M., & Nagaoka, A. (1992). Senescence-Accelerated Mouse (SAM): Age-Related Reduced Anxiety-Like Behavior in the SAM-P / 8 Strain, *51*(17), 979–985.

Momand, J. R., Xu, G., & Walter, C. A. (2013). Minireview The Paternal Age Effect : A Multifaceted Phenomenon, *88*(March), 1–9. https://doi.org/10.1095/biolreprod.112.103440

Monaghan, P., Maklakov, A. A., & Metcalfe, N. B. (2020). Intergenerational Transfer of Ageing: Parental Age and Offspring Lifespan. *Trends in Ecology and Evolution*, *35*(10), 927–937. https://doi.org/10.1016/j.tree.2020.07.005

Murgatroyd, C. A., Peña, C. J., Podda, G., Nestler, E. J., & Nephew, B. C. (2015). Early life social stress induced changes in depression and anxiety associated neural pathways which are correlated with impaired maternal care. *Neuropeptides*, *52*(3), 103–111. https://doi.org/10.1016/j.npep.2015.05.002

Nguyen, C. T. Y., Zhao, M., & Saltzman, W. (2020). Effects of sex and age on parental motivation in adult virgin California mice. *Behavioural Processes*, *178*(June), 104185. https://doi.org/10.1016/j.beproc.2020.104185

Ohl, F. (2003). Testing for anxiety. *Clinical Neuroscience Research*, *3*(4–5), 233–238. https://doi.org/10.1016/S1566-2772(03)00084-7

Ottinger, M. A. (2010). Mechanisms of reproductive aging: conserved mechanisms and environmental factors. *Annals of the New York Academy of Sciences*, *1204*(1), 73–81. https://doi.org/10.1111/j.1749-6632.2010.05653.x

Perea-Rodriguez, J. P., Zhao, M., Harris, B. N., Raqueno, J., & Saltzman, W. (2018). Behavioral and endocrine consequences of placentophagia in male California mice (Peromyscus californicus). *Physiology & Behavior*, *188*(November 2017), 283–290. https://doi.org/10.1016/j.physbeh.2018.02.022

Pike, K. N., Ashton, B. J., Morgan, K. V, & Ridley, A. R. (2019). Social and Individual Factors Influence Variation in Offspring Care in the Cooperatively Breeding Western Australian Magpie, *7*(April), 1–13. https://doi.org/10.3389/fevo.2019.00092

Pilakouta, N., Hanlon, E. J. H., & Smiseth, P. T. (2018). Biparental care is more than the sum of its parts: Experimental evidence for synergistic effects on offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884), 1–7. https://doi.org/10.1098/rspb.2018.0875

Pillay, N., & Rymer, T. L. (2015). Alloparenting enhances the emotional, social and cognitive performance of female African striped mice, Rhabdomys pumilio. *Animal Behaviour*, *99*, 43–52. https://doi.org/10.1016/j.anbehav.2014.10.003

R Core Team. (2023). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Reale, D., & Bousses, P. (1995). Effect of ewe age and high population density on the early nursing behaviour of mouflon. *Ethology Ecology and Evolution*, *7*(4), 323–334. https://doi.org/10.1080/08927014.1995.9522940

Reeve, H. K. (1992). Queen activation of lazy workers in colonies of the eusocial naked mole-rat, *358*(July), 147–149.

Ribble, D. O. (1990). *Population and social dynamics of the California mouse (Peromyscus californicus)*. University of California, Berkeley.

Ribble, D. O. (1991). The monogamous mating system of Peromyscus californicus as revealed by DNA fingerprinting. *Behavioral Ecology and Sociobiology*, 161–166. https://doi.org/10.1007/BF00166397

Ribble, D. O. (1992a). Dispersal in a monogamous rodent, Peromyscus californicus. *Ecology*, *73*(3), 859–866. https://doi.org/10.2307/1940163

Ribble, D. O. (1992b). Lifetime reproductive success and its correlates in the monogamous rodent, Peromyscus californicus. *Journal of Animal Ecology*, *61*(2), 457–468. https://doi.org/10.2307/5336

Ribble, D. O., & Salvioni, M. (1990). Social Organization and Nest Co-Occupancy in Peromyscus californicus, a monogamous rodent, 9–15.

Riedman, M. L. (1982). The Evolution of Alloparental Care and Adoption in Mammals and Birds. *The Quarterly Review of Biology*, *57*(4), 405–435. https://doi.org/10.1086/412936

Robitzsch, A., Grund, S., Henke, T., & Robitzsch, M. A. (2017). Package 'miceadds.' *Vienna: R Foundation for Statistical Computing*.

Rödel, H. G., Meyer, S., Prager, G., Stefanski, V., & Hudson, R. (2010). Litter size is negatively correlated with corticosterone levels in weanling and juvenile laboratory rats. *Physiology and Behavior*, *99*(5), 644–650. https://doi.org/10.1016/j.physbeh.2010.01.032

Rödel, H. G., Von Holst, D., & Kraus, C. (2009). Family legacies: Short- and long-term fitness consequences of early-life conditions in female European rabbits. *Journal of Animal Ecology*, *78*(4), 789–797. https://doi.org/10.1111/j.1365-2656.2009.01537.x

Rogers, F. D., Rhemtulla, M., Ferrer, E., & Bales, K. L. (2018). Longitudinal trajectories and inter-parental dynamics of prairie vole biparental care. *Frontiers in Ecology and Evolution*, *6*(JUN), 1–13. https://doi.org/10.3389/fevo.2018.00073

Rosenbaum, S., & Gettler, L. T. (2018). Physiology & Behavior With a little help from her friends (and family) part I : the ecology and evolution of non-maternal care in mammals. *Physiology & Behavior*, *193*(November 2017), 1–11. https://doi.org/10.1016/j.physbeh.2017.12.025

Royle, N. J., Smiseth, P. T., & Kölliker, M. (2012). *The evolution of parental care*. Oxford University Press.

Ryan, B. C., & Vandenbergh, J. G. (2002). Intrauterine position effects. *Neuroscience and Biobehavioral Reviews*, *26*(6), 665–678. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12479841

Salo, A. L., & French, J. A. (1989). Early experience, reproductive success, and development of parental behaviour in Mongolian gerbils. *Animal Behaviour*, *38*(4), 693–702. https://doi.org/10.1016/S0003-3472(89)80015-6

Sasvári, L., Hegyi, Z., Csörgõ, T., & Hahn, I. (2000). Age-dependent diet change, parental care and reproductive cost in tawny owls Strix aluco. *Acta Oecologica*, *21*(4–5), 267–275. https://doi.org/10.1016/S1146-609X(00)01080-8

Scheibler, E., Weinandy, R., & Gattermann, R. (2005). Intra-family aggression and offspring expulsion in Mongolian gerbils (Meriones unguiculatus) under restricted environments. *Mammalian Biology*, *70*(3), 137–146. https://doi.org/10.1016/j.mambio.2004.12.002

Sheldon, B. C. (2000). Differential allocation: Tests, mechanisms and implications. *Trends in Ecology and Evolution*, *15*(10), 397–402. https://doi.org/10.1016/S0169-5347(00)01953-4

Smith, P. T., Kolliker, M., & Royle, N. J. (2012). What is parental care. *The Evolution of Parental Care*, 1–14.

Smith, R. G., Kember, R. L., Mill, J., Fernandes, C., Schalkwyk, L. C., Buxbaum, J. D., & Reichenberg, A. (2009). Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: A mouse model. *PLoS ONE*, *4*(12), 4–8. https://doi.org/10.1371/journal.pone.0008456

Snowdon, C. T., & Pickhard, J. J. (1999). Family feuds: Severe aggression among cooperatively breeding cotton-top tamarins. *International Journal of Primatology*, *20*(5), 651–663. https://doi.org/10.1023/A:1020796517550

Snowdon, C. T., & Ziegler, T. E. (2007). Growing up cooperatively: family processes and infant care in marmosets and tamarins. *Journal of Developmental Processes*, *2*(1), 40–66.

Sparkman, A. M., Blois, M., Adams, J., Waits, L., Miller, D. A. W., & Murray, D. L. (n.d.). Evidence for sex-specific reproductive senescence in monogamous cooperatively breeding red wolves. https://doi.org/10.1007/s00265-016-2241-9

Stead, S., Mucha, S., & Bădescu, I. (2019). Alloparental Care. *Encyclopedia of Animal Cognition and Behavior*, (Wisenden 1999), 1–7. https://doi.org/10.1007/978-3-319-47829-6_1400-1

Stiver, K. A., & Alonzo, S. H. (2011). Alloparental care increases mating success. *Behavioral Ecology*, *22*(1), 206–211. https://doi.org/10.1093/beheco/arq186

Stolzenberg, D. S., & Champagne, F. A. (2016). Hormonal and non-hormonal bases of maternal behavior: The role of experience and epigenetic mechanisms. *Hormones and Behavior*, *77*, 204–210. https://doi.org/10.1016/j.yhbeh.2015.07.005

Stone, A. I., Mathieu, D., Griffin, L., & Bales, K. L. (2010). Alloparenting experience affects future parental behavior and reproductive success in prairie voles (Microtus ochrogaster). *Behavioural Processes*, *83*(1), 8–15. https://doi.org/10.1016/j.beproc.2009.08.008

Storey, A. E., & Walsh, C. J. (2013). Biological basis of mammalian paternal behavior. *Handbook of Father Involvement: Multidisciplinary Perspectives*, *2*, 3–22.

Swartz, K. B., & Rosenblum, L. A. (1981). The social context of parental behavior. In *Parental care in mammals* (pp. 417–454). Springer.

Tidière, M., Thevenot, X., Deligiannopoulou, A., Douay, G., Whipple, M., Siberchicot, A., … Lemaître, J. F. (2018). Maternal reproductive senescence shapes the fitness consequences of the parental age difference in ruffed lemurs. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1886). https://doi.org/10.1098/rspb.2018.1479

Trainor, B. C., & Marler, C. A. (2001). Testosterone, paternal behavior, and aggression in the monogamous California mouse (Peromyscus californicus). *Hormones and Behavior*, *40*(1), 32–42. https://doi.org/10.1006/hbeh.2001.1652

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In R. L. Trivers (Ed.), *Sexual Selection and the Descent of Man* (pp. 136–179). Routledge. https://doi.org/10.4324/9781315129266-7

Trivers, R. L. (1974). Parent-Offspring Conflict. *American Zoologist*, *14*(1), 249–264. https://doi.org/10.1093/icb/14.1.249

van Buuren, S., & Groothuis-Oudshoorn, K. (2011). mice: Multivariate imputation by chained equations in R. *Journal of Statistical Software*, *45*(3), 1–67. https://doi.org/10.18637/jss.v045.i03

Vleck, C. M., Vleck, D., & Palacios, M. G. (2011). Evolutionary ecology of senescence: A case study using tree Swallows, Tachycineta bicolor. *Journal of Ornithology*. https://doi.org/10.1007/s10336-010-0629-2

Wilcoxen, T. E., Boughton, R. K., & Schoech, S. J. (2010). Older can be better: Physiological costs of paternal investment in the Florida scrub-jay. *Behavioral Ecology and Sociobiology*, *64*(10), 1527–1535. https://doi.org/10.1007/s00265-010-0966-4

Wittenberger, J. F., & Tilson, R. L. (1980). The evolution of monogamy: hypotheses and evidence. *Annual Review of Ecology and Systematics*, *11*(1), 197–232.

Woodroffe, R., & Vincent, A. (1994). Mother' s little helpers: patterns of male care in mammals, (8), 294–297.

Wu, R., Song, Z., Tai, F., An, X., Yu, P., & Li, Y. (2013). The effect of alloparental experience and care on anxiety-like, social and parental behaviour in adult mandarin voles. *Animal Behaviour*, *85*(1), 61–69. https://doi.org/10.1016/j.anbehav.2012.10.007

Wu, R., Song, Z., Tai, F., Wang, L., Kong, L., & Wang, J. (2013). Post-weaning living with parents during juvenile period alters locomotor activity, social and parental behaviors in mandarin voles. *Behavioural Processes*, *98*, 78–84. https://doi.org/10.1016/j.beproc.2013.05.008

Xie, K., Ryan, D. P., Pearson, B. L., Henzel, K. S., Neff, F., Vidal, R. O., … Ehninger, D. (2018). Epigenetic alterations in longevity regulators, reduced life span, and exacerbated aging-related pathology in old father offspring mice. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(10), E2348–E2357. https://doi.org/10.1073/pnas.1707337115

Zhao, M., Garland, T., Chappell, M. A., Andrew, J. R., Saltzman, W., Garland Jr, T., … Saltzman, W. (2017). Metabolic and affective consequences of fatherhood in male California mice. *Physiology and Behavior*, *177*(April), 57–67. https://doi.org/10.1016/j.physbeh.2017.04.010

Ziegler, T. E. (2000). Hormones associated with non-maternal infant care: A review of mammalian and avian studies. *Folia Primatologica*, *71*(1–2), 6–21. https://doi.org/10.1159/000021726

Zöttl, M., Fischer, S., & Taborsky, M. (2013). Partial brood care compensation by female breeders in response to experimental manipulation of alloparental care. *Animal Behaviour*, *85*(6), 1471–1478. https://doi.org/10.1016/j.anbehav.2013.03.045

Table 3.1. Counts of focal pups (L1, L1+, L2, L2+) and litters

	L1	**L2**	**L1+**	**L2+**	**Total**
Male	13	8	18	8	47
Female	12	20	7	25	64
Total	25	28	25	33	111
Litters	13	12	13	13	51

Table 3.2. Count of animals that behaved parentally, attacked, or behaved neutrally (e.g., neither parentally nor attacked) during the parental-behavior test.

		L1	**L1+**	**L2**	**L2+**
Females	Neutral	5	3	7	6
	Parental	3	2	1	5
	Attack	1	0	1	3
Males	Neutral	3	4	2	0
	Parental	6	10	3	3
	Attack	1	1	2	0

Table 3.3. Mothers' body mass, fat mass, and lean mass (not transformed; mean ± SE) and results of repeated-measures ANOVAs calculated with the natural logarithm of body, fat, and lean mass. ANOVAs for fat and lean mass include body mass as a covariate. Week numbers refer to number of weeks since the birth of the second litter. p-values <0.05 are in bold.

	Body Mass (g)			**Fat Mass (g)**			**Lean Mass (g)**		
Week	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**
Litter 1 (n = 12)	42.4 ± 1.9	43.4 ± 1.5	47.1 ± 2.2	5.52 ± 0.8	6.11 ± 0.7	6.78 ± 0.6	33.9 ± 1.5	33.4 ± 1.0	36.8 ± 1.7
Litter 2 (n = 8)	44.9 ± 2.2	46.5 ± 1.8	50.1 ± 2.4	5.61 ± 0.9	5.10 ± 0.7	5.37 ± 0.7	32.2 ± 4.3	37.8 ± 1.7	39.9 ± 1.6
Overlap. Litters (n = 13)	47.2 ± 1.5	48.3 ± 1.1	50.6 ± 2.0	9.49 ± 2.3	9.05 ± 2.2	9.30 ± 2.3	33.7 ± 2.5	34.6 ± 2.5	35.7 ± 2.6

	F	**p**	**F**	**p**	**F**	**P**
Family Type	5.75	**< 0.01**	1.25	0.30	0.84	0.44
Week	4.41	**0.02**	1.26	0.29	0.18	0.83
Family Type * Week	0.11	0.98	2.01	0.11	1.90	0.12

Table 3.4. Fathers’ body mass and body composition (not transformed; mean ± SE) and results of repeated-measures ANOVAs calculated with the natural logarithm of body, fat, and lean mass. ANOVAs for fat and lean mass include body mass as a covariate. Week numbers refer to number of weeks since the birth of the second litter. Fathers did not significantly differ on any measure based on family type.

	Body Mass (g)			**Fat Mass (g)**			**Lean Mass (g)**		
Week	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**
Litter 1 (n = 12)	39.9 ± 1.6	40.4 ± 1.8	41.5 ± 2.0	8.60 ± 7.2	8.49 ± 7.1	8.68 ± 7.1	28.1 ± 7.8	27.7 ± 7.7	28.5 ± 7.8
Litter 2 (n = 8)	42.3 ± 2.6	43.7 ± 2.9	44.6 ± 2.8	7.85 ± 4.8	8.05 ± 4.7	8.82 ± 4.7	32.3 ± 3.1	32.7 ± 4.2	32.6 ± 4.0
Overap. Litters (n = 13)	41.1 ± 2.4	41.4 ± 2.4	41.2 ± 2.5	9.14 ± 8.0	9.22 ± 7.8	10.8 ± 9.9	28.0 ± 7.9	28.2 ± 7.7	26.8 ± 9.2
	F		**p**	**F**		**p**	**F**		**P**
Family Type	1.15		0.321	0.35		0.71	0.35		0.71
Week	0.25		0.782	0.06		0.94	0.06		0.94
Family Type * Week	0.07		0.992	0.90		0.47	0.90		0.47

Table 3.5. Body mass and body composition (not transformed; mean ± SE) of older offspring (L1/L1+) and results of Type III Ward tests from linear mixed models calculated with the natural logarithm of body, fat, and lean mass. Models for fat and lean mass include body mass as a fixed effect. Week numbers refer to number of weeks since the birth of the second litter. p-values <0.05 are in bold, and p-values > 0.05 and < 0.10 are italicized.

	Body Mass (g)				**Fat Mass (g)**				**Lean Mass (g)**			
Week	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
L1 Females n = 11	22.5 ± 1.3	26.0 ± 1.4	28.4 ± 1.4	30.3 ± 1.4	2.66 ± 0.3	3.28 ± 0.5	4.19 ± 0.7	4.92 ± 0.9	18.6 ± 1.1	21.2 ± 0.9	22.2 ± 0.7	23.6 ± 0.7
L1 Males n = 12	22.9 ± 1.2	24.9 ± 1.1	28. 0 ± 1.0	28.9 ± 0.9	2.56 ± 0.2	2.93 ± 0.4	3.61 ± 0.5	3.62 ± 0.5	18.3 ± 1.0	20.7 ± 0.6	22.0 ± 0.6	23.3 ± 0.6
L1 + Females n = 7	23.3 ± 0.9	25.8 ± 1.0	29.0 ± 1.6	25.4 ± 0.8	2.74 ± 0.4	3.38 ± 0.6	4.15 ± 0.6	3.23 ± 0.3	19.2 ± 0.6	20.6 ± 0.5	21.0 ± 0.7	20.5 ± 0.6
L1+ Males n = 13	24.8 ± 1.1	26.8 ± 0.5	28.2 ± 0.6	29.2 ± 0.7	2.45 ± 0.2	2.91 ± 0.2	3.15 ± 0.3	3.51 ± 0.4	20.4 ± 0.4	22.2 ± 0.5	23.0 ± 0.5	23.2 ± 0.5
		X^2	**P**			**X^2**	**P**			**X^2**	**P**	
Family Type		1.21	0.27			0.27	0.61			0.01	0.92	
Sex		0.23	0.63			3.63	*0.06*			3.44	*0.067*	
Week		74.97	**< 0.01**			0.57	0.63			6.41	**< 0.01**	
Litter Type * Sex		0.12	0.73			1.99	0.16			2.09	0.15	
Litter Type * Week		16.53	**< 0.01**			0.23	0.88			2.09	0.10	
Sex * Week		1.47	0.69			0.28	0.84			1.21	0.31	
Litter Type * Sex * Week		4.08	0.25			0.24	0.87			0.77	0.51	

Table 3.6. Body mass and body composition (not transformed; mean ± SE) of younger offspring (L2/L2+) and results of Type III Ward tests from linear mixed models calculated with the natural logarithm of body, fat, and lean mass. Models for fat and lean mass include body mass as a fixed effect. p-values <0.05 are in bold, and p-values > 0.05 and < 0.10 are italicized.

	Body Mass (g)			**Fat Mass (g)**			**Lean Mass (g)**		
Week after birth of second litter	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**
L2 n = 19	9.7 ± 0.3	12.1 ± 0.3	16.6 ± 0.7	0.8 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	13.7 ± 5.2	10.4 ± 0.4	14.0 ± 0.6
L2+ n = 32	9.7 ± 0.3	11.8 ± 0.4	15.2 ± 0.5	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	8.5 ± 0.2	9.8 ± 0.3	13.2 ± 0.4

	X^2	**P**	**X^2**	**P**	**X^2**	**P**
Litter Type	0.34	0.56	0.54	0.462	3.32	*0.07*
Week	264.25	**< 0.01**	2.12	0.35	2.08	0.35
Litter Type * Week	2.19	0.33	7.00	**< 0.03**	2.11	0.35

Table 3.7. Behaviors (means ± SE) of older offspring (L1/L1+) in the open field and results of Type III Ward tests from generalized linear mixed models. p-values <0.05 are in bold.

	L1 Females n =10	**L1 Males** n= 13	**L1+ Females** n = 7	**L1+ Males** n=10	Treatment		Sex		Treatment * Sex	
					X^2	**P**	**X^2**	**P**	**X^2**	**P**
Duration in Edges of Arena (s)	567.4 ± 8.9	552.9 ± 11.4	560.9 ± 10.1	567.4 ± 6.8	0.18	0.67	1.96	0.16	0.93	0.34
Duration in Center of Arena (s)	47.9 ± 7.4	56.8 ± 9.4	35.1 ± 10.3	37.1 ± 6.6	0.90	0.34	0.36	0.55	0.00	0.96
Distance Traveled in Periphery (m)	53.23± 5.3	67.0 ± 4.2	45.6 ± 9.3	62.7 ± 4.4	3.84	**0.05**	0.82	0.37	0.02	0.89
Distance Traveled in Center (m)	8.3 ± 0.8	9.6 ± 0.9	51.1 ± 1.6	6.7 ± 1.3	1.00	0.32	4.54	**0.03**	0.12	0.73
Total Distance Traveled (m)	61.6 ± 5.7	76.6 ± 4.5	50.7 ± 10.4	69.4 ± 5.3	1.48	0.22	3.86	**0.05**	0.1	0.76

Table 3.8. Behaviors (means ± SE) of younger offspring (L2/L2+) in the open field and Type III Ward tests from generalized linear mixed models.

	L2 Females n = 19	**L2 Males** n = 7	**L2+ Females** n = 19	**L2+ Males** n = 5	Treatment		Sex		Treatment * Sex	
					X^2	**P**	**X^2**	**P**	**X^2**	**P**
Duration in Edges of Arena (s)	557.3 ± 11.2	555.7 ± 14.0	566.5 ± 6.4	574.2 ± 11.1	0.84	0.36	0.17	0.68	0.00	0.97
Duration in Center of Arena (s)	49.3 ± 7.8	46.2 ± 14.4	32.8 ± 5.3	27.3 ± 10.7	1.26	0.26	0.02	0.90	0.02	0.8
Distance Traveled in Periphery (m)	43.2 ± 3.2	48.1 ± 7.2	47.8 ± 5.4	56.4 ± 11.8	0.36	0.55	0.13	0.71	0.03	0.85
Distance Traveled in Center (m)	4.8 ± 0.7	5.9 ± 1.6	4.9 ± 0.7	7.2 ± 2.6	0.01	0.91	0.27	0.60	0.09	0.77
Total Distance Traveled (m)	48.0 ± 3.6	53.9 ± 8.1	52.7 ± 5.7	63.5 ± 14.1	0.38	0.54	0.31	0.58	0.05	0.82

Table 3.9. Behavior of female focal offspring in the parental-behavior test and results of Type III Wald $\chi2$ tests comparing litter types. We defined parental behavior as grooming the pup and/or huddling over the pup. All values for n represent the number of animals that performed a given behavior during the test. p-values < 0.05 are in bold.

		L1 Females		**L1+ Females**		**L2 Females**		**L2+ Females**		**Type III Wald $\chi2$ test**	
		n	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**$\chi2$**	**P**
Parental Behavior	Latency to Initiate Parental Behavior (s)	3	704.7 ± 246.6	2	292.9 ± 240.5	0	NA	5	748.3 ± 482.1	1.53	0.47
	Duration of Huddling Pup (s)	3	886.6 ± 659.8	2	1390.4 ± 1383.3	0	NA	5	1796.1 ± 747.9	0.50	0.78
	Duration of Grooming Pup (s)	1	865.7 ± NA	1	1804.4 ± NA	0	NA	3	1018.7 ± 477.1	NA	NA
	Total Duration of Parental Behavior (s)	3	1185.3 ± 628.5	2	2332.4 ± 2325.3	0	NA	5	2438.7 ± 1070.6	0.30	0.86
Other Pup-directed Behavior	Latency to Sniff Pup (s)	8	306.1 ± 129.1	3	139.7 ± 114.7	6	546.3 ± 431.8	13	172.6 ± 41.5	0.99	0.80
	Latency to Contact Pup (s)	5	913.4 ± 411.8	3	145.2 ± 113.3	1	77.5 ± NA	7	682.0 ± 465.5	4.84	0.18
	Duration of Sniffing Pup (s)	8	335.6 ± 231.9	3	85.3 ± 41.5	6	160.8 ± 146.3	13	113.1 ± 57.9	1.27	0.74
	Duration of Contact with Pup (s)	5	328.7 ± 195.9	3	391.3 ± 379.9	1	8.6 ± NA	7	113.1 ± 74.0	1.27	0.74

Table 3.9. cont.

		L1 Females		L1+ Females		L2 Females		L2+ Females		Type III Wald χ2 test	
		n	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**χ2**	**P**
Non-pup-directed Behavior	Duration of Locomotion (s)	9	456.6 ± 263.3	3	249.2 ± 202.1	6	216.6 ± 109.8	13	120.2 ± 35.0	1.39	0.71
	Duration of Eating (s)	3	33.6 ± 14.9	0	NA	2	119.2 ± 39.4	3	64.9 ± 25.6	NA	NA
	Duration of Autogrooming (s)	7	78.6 ± 25.1	3	106.8 ± 33.2	5	103.7 ± 19.6	9	90.5 ± 33.5	2.19	0.53
	Duration of Resting (s)	8	1378.1 ± 520.3	5	1179.6 ± 595.1	8	606.4 ±122.2	13	946.6 ± 341.0	0.79	0.85
	Duration of Manipulating Bedding (s)	2	15.2 ± 8.8	1	79.6 ± NA	2	64.3 ± 27.6	2	78.5 ± 32.2	NA	NA
	Total Duration of Non-pup-directed Behavior (s)	9	528.9 ± 253.9	4	267.0 ± 130.2	6	342.7 ± 141.8	14	183.7 ± 48.5	0.27	0.96

Table 3.10. Behavior of male focal offspring in the parental-behavior test and results of Type III Wald χ2 tests comparing L1 and L1+ males. L2 and L2+ males were not included in the analyses because we had data from too few animals. We defined parental behavior as grooming the pup and/or huddling over the pup. All values for n represent the number of animals that performed a given behavior during the test. p-values < 0.05 are in bold.

		L1 Males		L1+ Males		L2 Males		L2+ Males	Type III Wald χ2 test on L1/L1+		
		n	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**χ2**	**P**
Parental Behavior	Latency to Initiate Parental Behavior (s)	4	165.3 ± 61.5	6	131.2 ± 20.8	3	620.7 ± 496.2	2	175.9 ± 110.3	0.14	0.71
	Duration of Huddling Pup (s)	4	1533.4 ± 571.8	6	1719.5 ± 582.5	1	2160.4 ± NA	2	2713.1 ± 495.6	0.14	0.71
	Duration of Grooming Pup (s)	4	790.0 ± 496.7	4	796.2 ± 435.9	3	366.7 ± 349.8	1	1030.4 ± NA	0.05	0.82
	Total Duration of Parental Behavior (s)	4	2391.9 ± 623.6	6	2295.5 ± 739.6	3	1181.5 ± 1152.9	2	3235.6 ± 12.3	0.11	**0.74**
Other Pup-directed Behavior	Latency to Sniff Pup (s)	10	216.4 ± 89.4	13	69.8 ±17.2	7	400.1 ± 189.5	3	64.1 ± 19.2	4.88	**0.03**
	Latency to Contact Pup (s)	8	563.5 ± 261.6	12	187.6 ± 95.8	4	308.2 ± 184.6	3	150.0 ± 72.3	4.42	**0.04**
	Duration of Sniffing Pup (s)	10	52.7 ± 6 10.6	13	112.8 ± 39.3	7	156.8 ± 60.6	3	126.3 ± 30.5	2.57	0.11
	Duration of Contact with Pup (s)	8	481.0 ± 444.1	12	776.5 ± 0.1	3	33.8 ± 23.5	2	65.5 ± 60.3	1.37	0.24

Table 3.10. cont.

		L1 Males		L1+ Males		L2 Males		L2+ Males		Type III Wald χ2 test on L1/L1+	
		n	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**n**	**Mean ± SE**	**χ2**	**P**
Non-pup-directed Behavior	Duration of Locomotion (s)	3	266.3 ± 123.2	7	351.3 ± 98.5	5	447.0 ± 159.0	3	193. 4 ± 110. 4	0.00	0.95
	Duration of Eating (s)	3	25.3 ± 9.7	7	29.2 ± 13.1	3	40.9 ± 6.5	0	NA	0.54	0.46
	Duration of Autogrooming (s)	7	49.5 ± 23.8	10	99.0 ± 26.5	5	120.2 ± 63.5	1	33.1 ± NA	0.00	0.97
	Duration of Resting (s)	10	1222.9 ± 391.4	13	1172.3 ± 375.0	6	353.2 ± 122.5	3	131.8 ± 93.2	0.10	0.76
	Duration of Manipulating Bedding (s)	4	114.4 ± 22.1	8	40.2 ± 27.5	3	106.7 ± 77.0	2	31.1 ± 16.5	10.20	**< 0.01**
	Total Duration of Non-pup-directed Behavior (s)	9	163.6 ± 61.1	14	312.7 ± 68.5	6	493.1 ± 144.5	3	204.4 ± 120.8	1.54	0.22

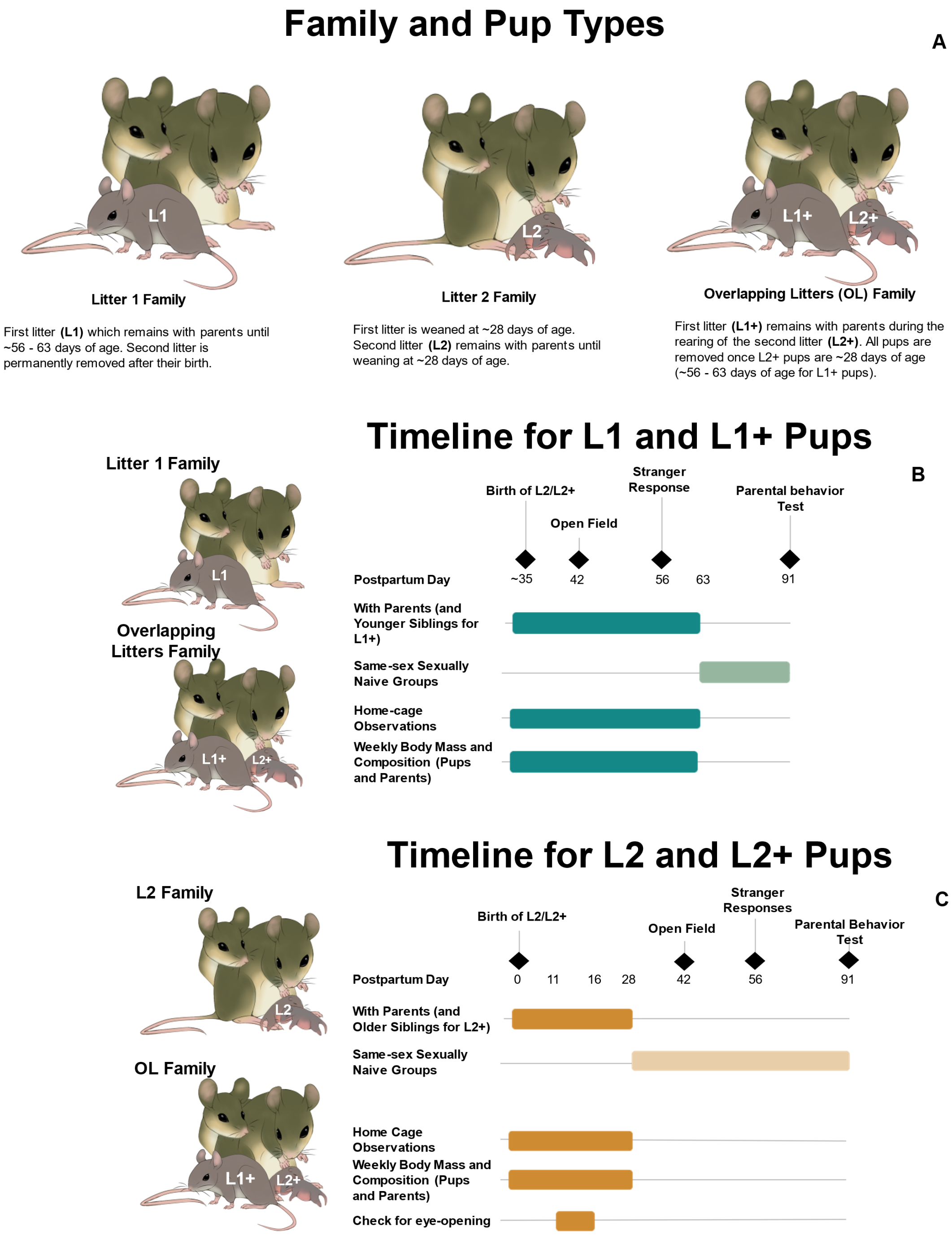

Figure 3.1. Experimental Design and Timeline. (A) Description of Litter 1, Litter 2, and Overlapping Litters families and focal offspring. Experimental timelines for (B) older pups (L1 and L1+) and (C) younger pups (L2 and L2+).

Figure 3.2. Double-cage setup. Both cages contained food, water, bedding, and nesting material, and animals could move freely between the two cages at all times.

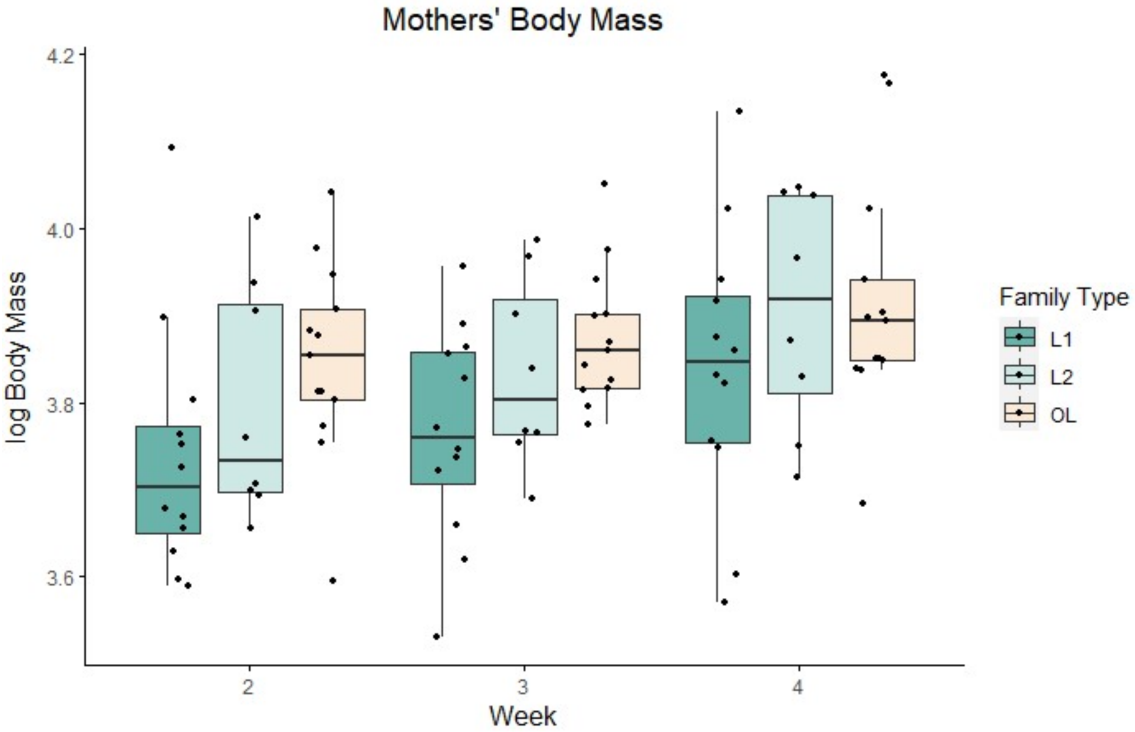

Figure 3.3. Log-transformed body masses of mothers in Litter 1 (L1, n = 12), Litter 2 (L2, n = 8), and Overlapping Litters families (OL, n =13). Circles represent data from individual animals, horizontal lines represent means, and error bars represent 1.5*Interquartile range + first and third quartile. OL mothers had significantly higher body mass than L1 mothers ($p < 0.01$).

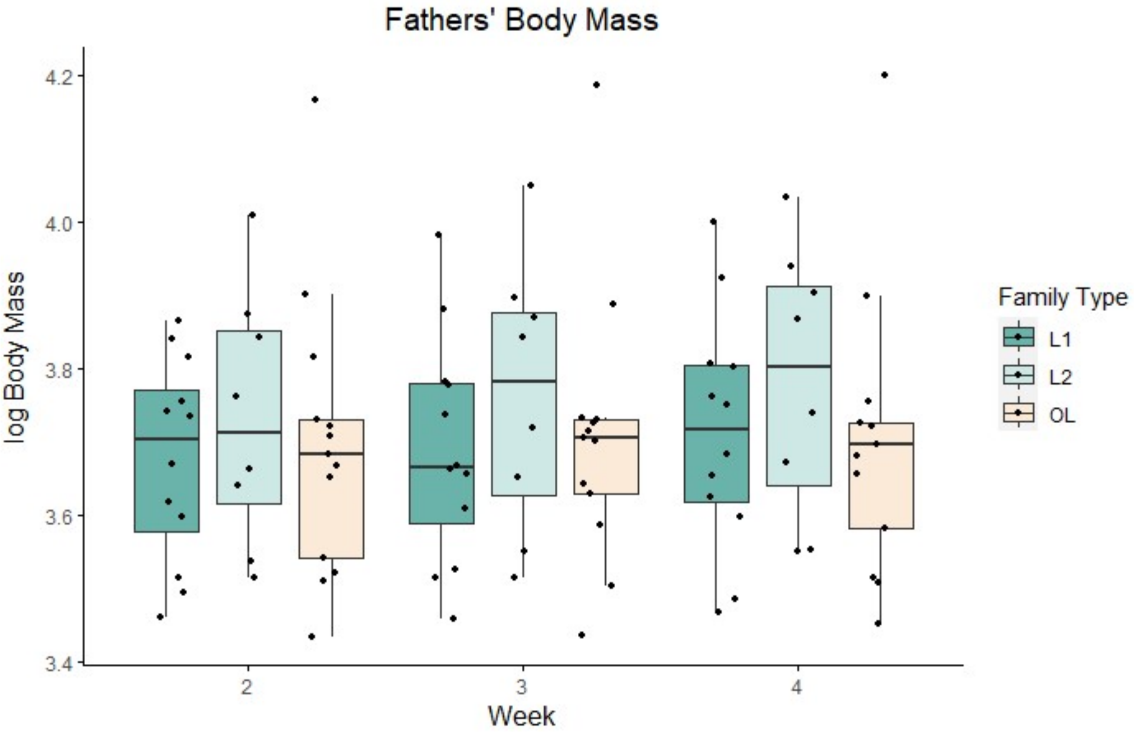

Figure 3.4. Log-transformed body masses of fathers in Litter 1 (L1, n = 12), Litter 2 (L2, n = 8), and Overlappping Litters families (OL, n =13). Circles represent data from individual animals, horizontal lines represent means, and error bars represent 1.5*Interquartile range + first and third quartile. Fathers from different family types did not significantly differ in body mass.

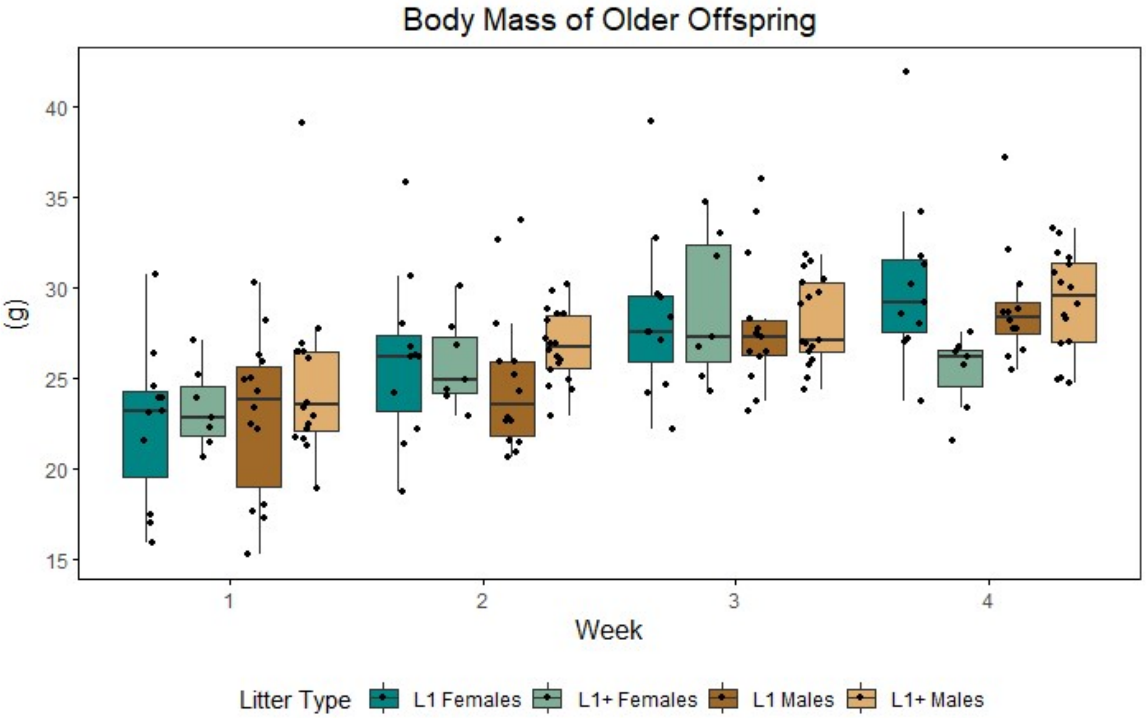

Figure 3.5. Body masses of L1 females (n = 11), L1+ females (n = 7), L1 males (n = 12), and L1+ males (n = 13) across the four weeks following the birth of the next litter. Circles represent data from individual animals, horizontal lines represent means, and error bars represent 1.5*Interquartile range + first and third quartile. Body mass of L1 offspring increased significantly between weeks 1 and 2 ($p < 0.01$) and between weeks 2 and 3 ($p < 0.01$). Body mass of L1+ offspring increased significantly between weeks 1 and 2 ($p = 0.03$).

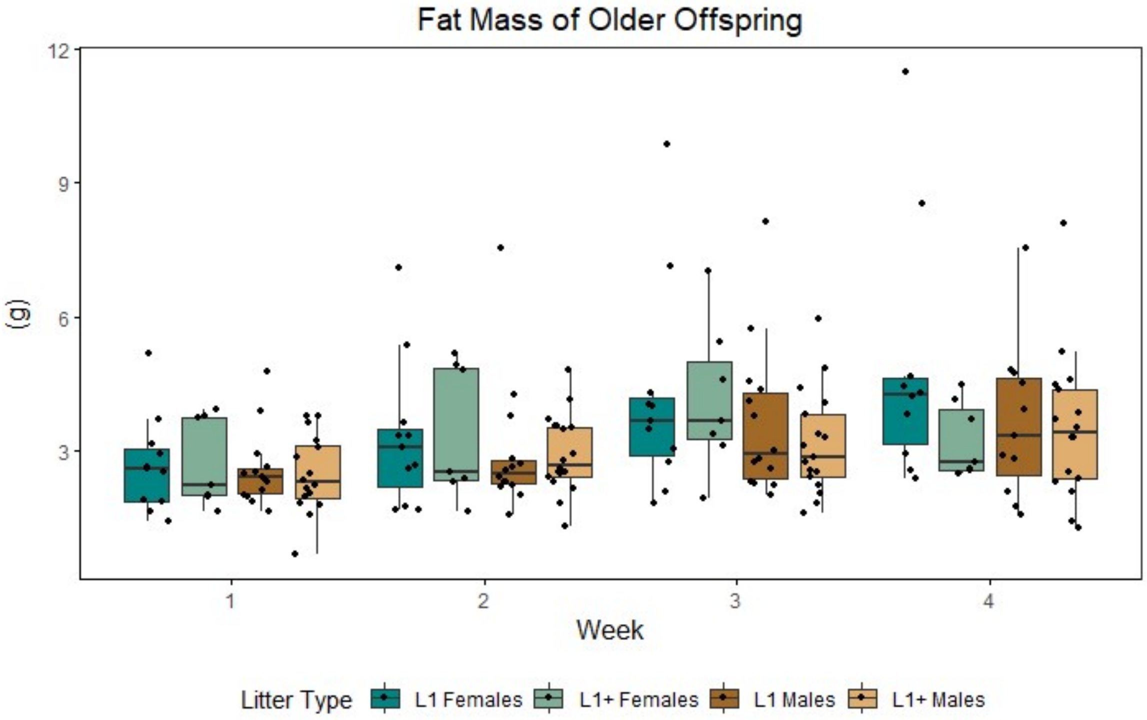

Figure 3.6. Fat masses of L1 females (n = 11), L1+ females (n = 7), L1 males (n = 12), and L1+ males (n = 13). Circles represent data from individual animals. Older offspring from different litter types did not significantly differ in fat mass. Females tended to have higher fat mass than males (p = 0.07).

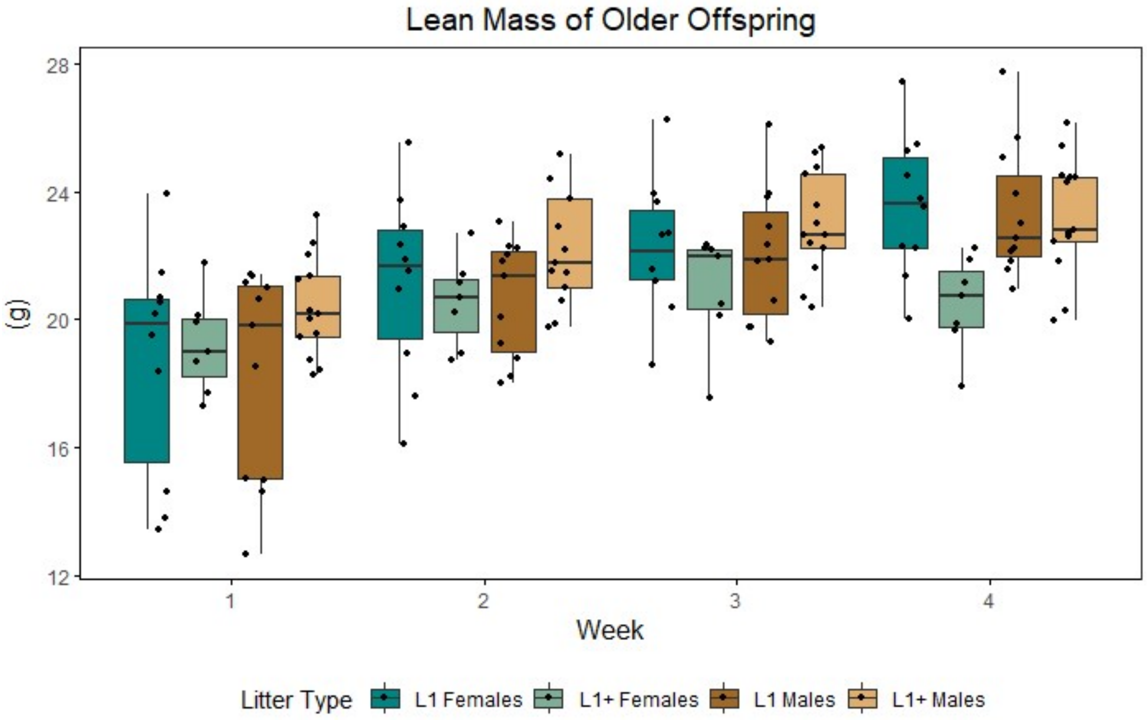

Figure 3.7. Lean masses of L1 females (n = 11), L1+ females (n = 7), L1 males (n = 12), and L1+ males (n = 13). Circles represent data from individual animals. Males tended to have higher lean mass than females ($p = 0.07$).

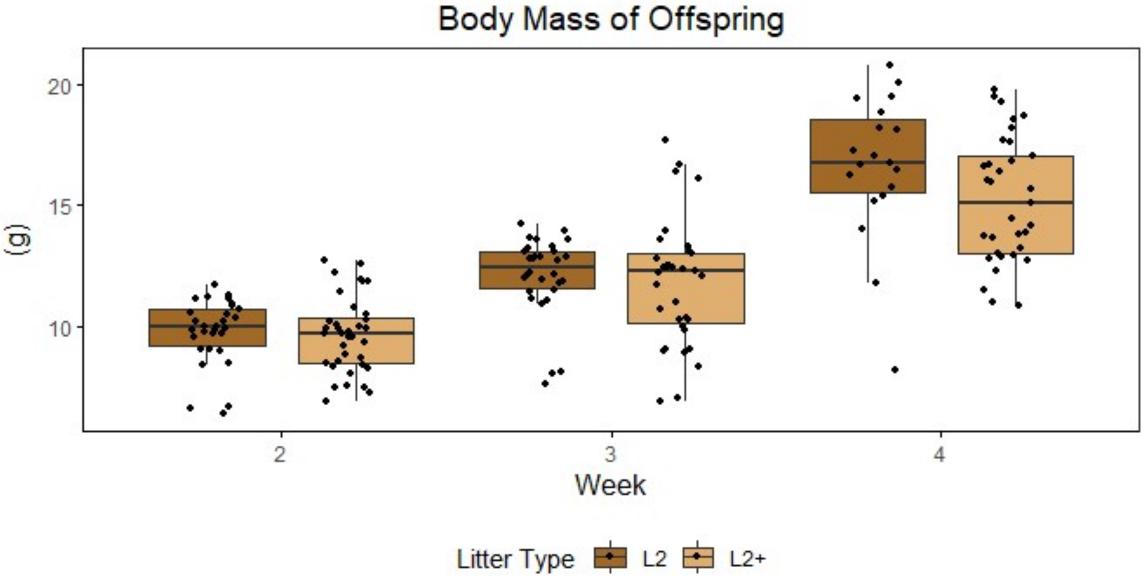

Figure 3.8. Body masses of L2 (n = 19) and L2+ (n = 32) focal offspring. Males and females were not analyzed separately because we were unable to determine sex until after weaning. Circles represent data from individual animals. Body mass increased weekly in younger offspring ($p < 0.01$).

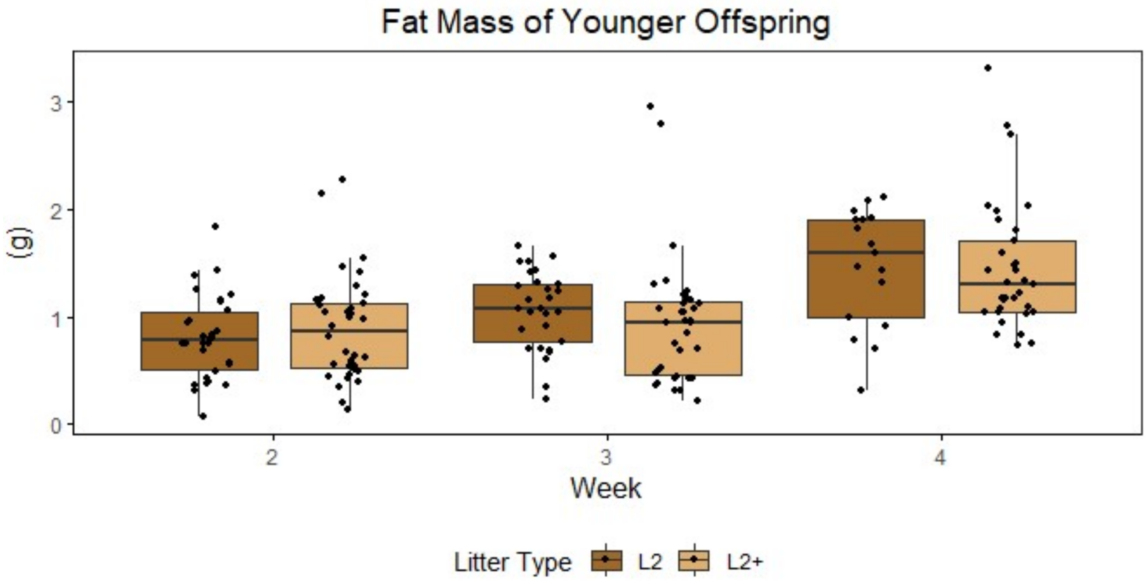

Figure 3 9. Fat masses of L2 (n = 19) and L2+ (n = 32) focal offspring. Males and females were not analyzed separately because we were unable to determine sex until after weaning. Circles represent data from individual animals. L2+ offspring, but not L2 offspring, showed a significant gain in fat mass between Weeks 2 and 3 (p = 0.04).

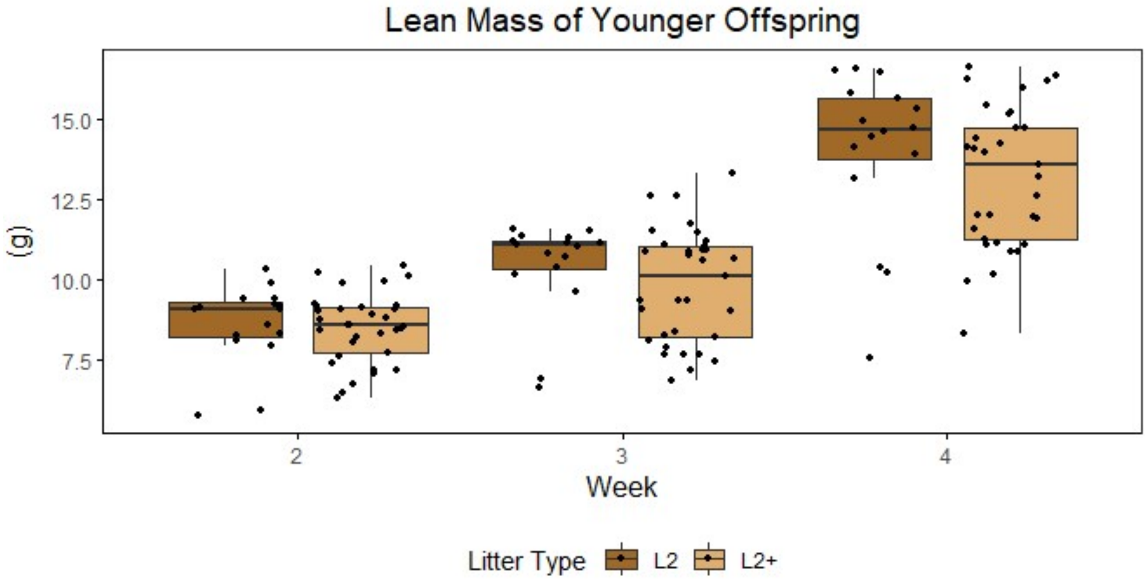

Figure 3.10. Lean masses of L2 (n = 19) and L2+ (n = 32) focal offspring. Males and females were not analyzed separately because we were unable to determine sex until after weaning. Circles represent data from individual animals. Neither litter type nor week significantly affected lean mass in younger offspring.

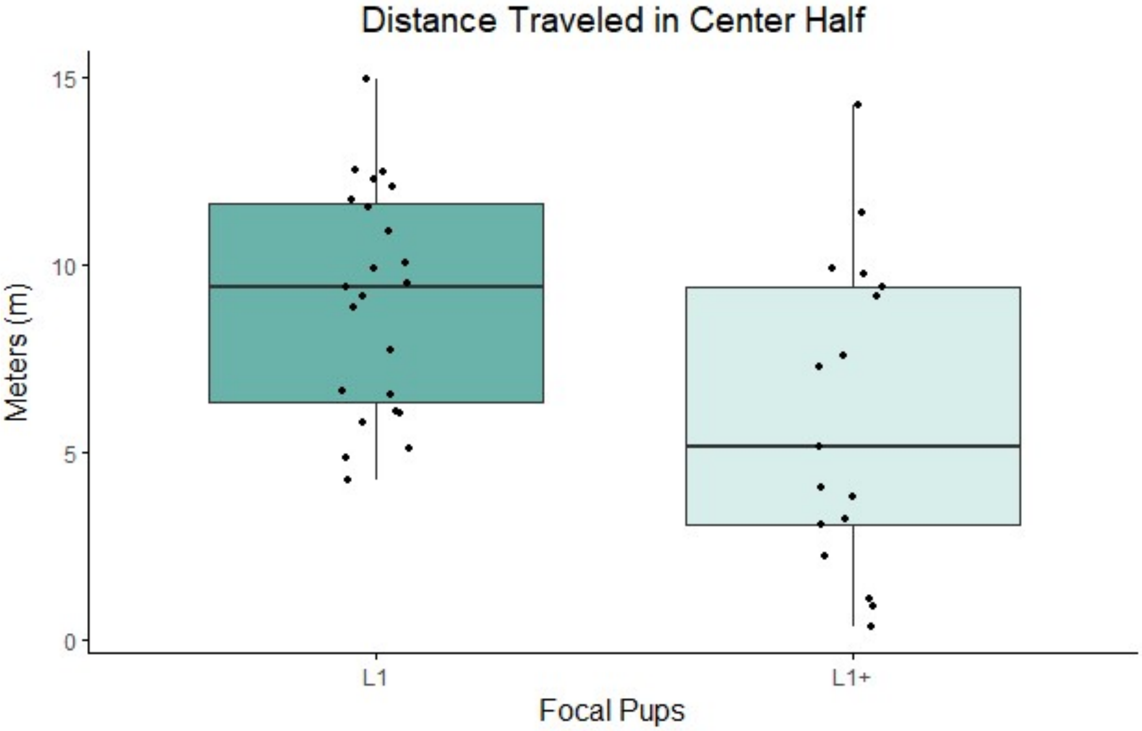

Figure 3.11. Distance traveled in the center of the open field. Circles represent data from individual animals. L1 offspring traveled greater distances in the center of the open field than L1+ offspring (p = 0.05).

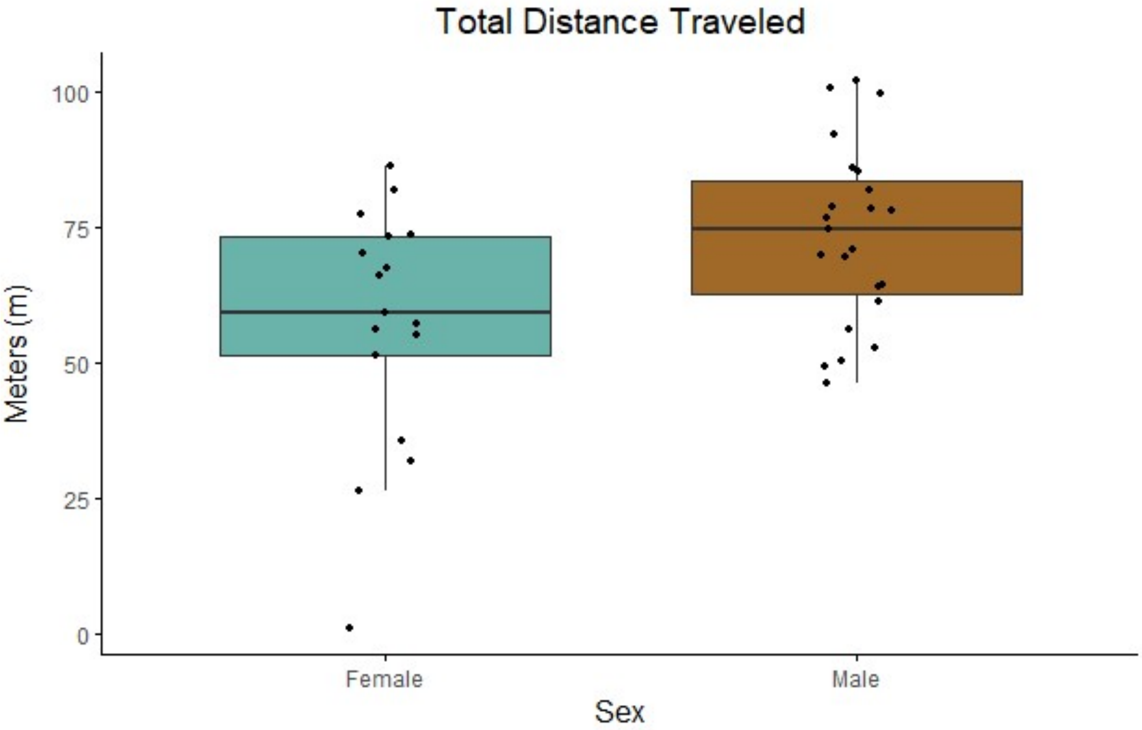

Figure 3.12. Total distance traveled in the open field by older offspring. Circles represent data from individual animals. Males traveled greater distances than females (p = 0.03).

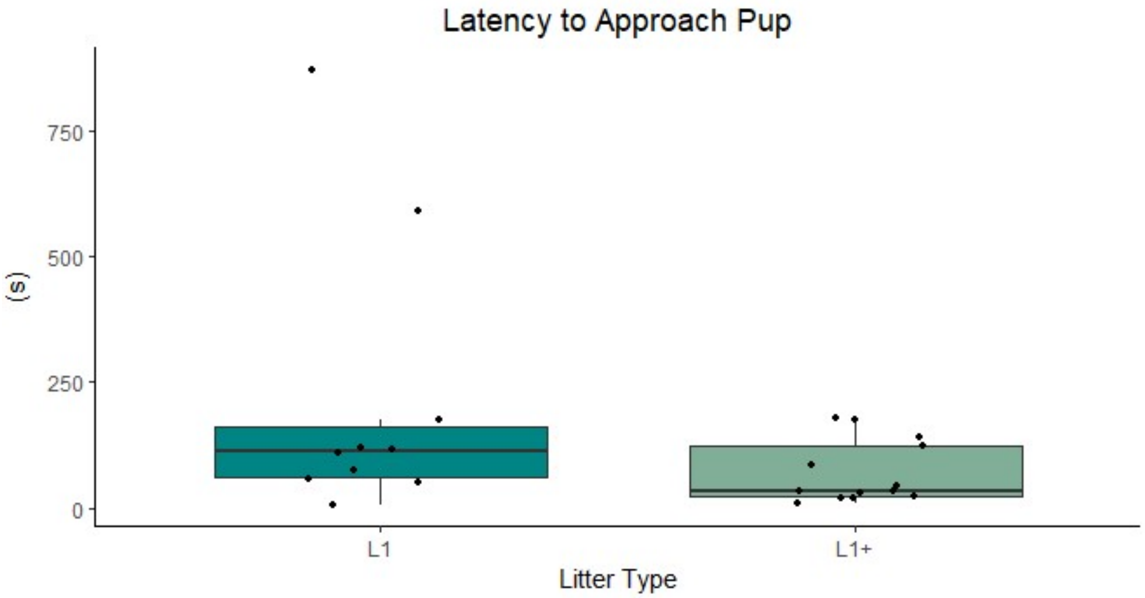

Figure 3.13. Latencies of L1 (n = 10) and L1+ (n = 13) males to approach the pup during the parental-behavior test. Circles represent data from individual animals. L1+ males were faster to approach than L1 males (p = 0.03).

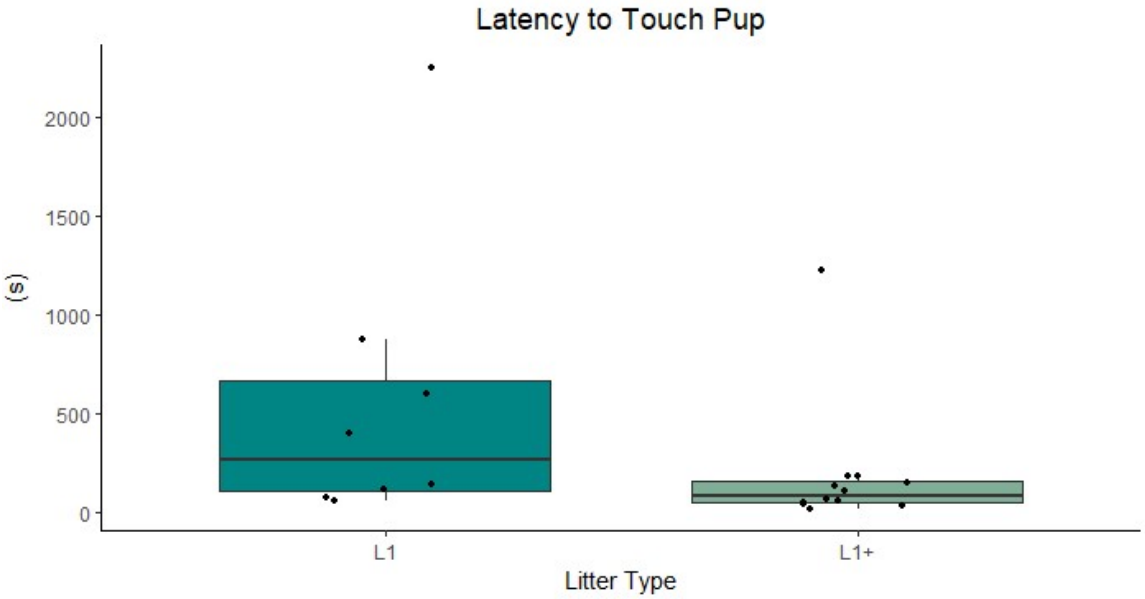

Figure 3.14. Latencies of L1 (n = 8) and L1+ (n = 13) males to touch the pup during the parental behavior test. Circles represent data from individual animals. L1+ males were faster to approach than L1 males (p = 0.04).

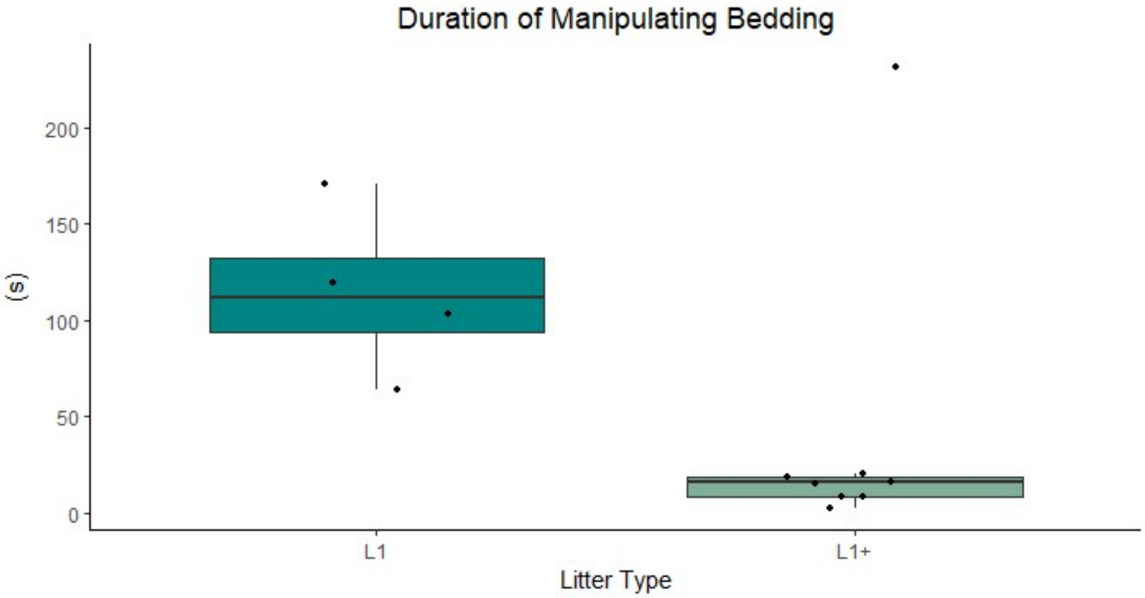

Figure 3.15. Duration of manipulating bedding by L1 (n = 4) and L1+ (n = 8) males during the parental-behavior test. Circles represent data from individual animals. L1 males manipulated shavings more than L1+ males ($p < 0.01$).

Synthesis

Much of our understanding of parental care has focused on either intrinsic characteristics of an individual or its surrounding environment. My dissertation takes both into account by examining how both characteristics intrinsic to an individual and social environment can affect caregiving behavior in the biparental, monogamous California mouse. I first uncovered evidence for sex-specific patterns of offspring discrimination in this species across the pup-rearing period; fathers showed increased interest in unrelated pups during the peak periods of parental care while mothers did not (Chapter 1). In addition to the effects of individual traits or social interactions alone, the amount and/or quality of care provided by one animal may influence how other animals provide care. I examined the effects of late paternity, not only on the behaviors of fathers, but also on those of their mates (Chapter 2). Although no difference in paternal behavior or offspring development were observed between old and young fathers, mothers mated to young fathers nursed pups more frequently than those mated to old fathers. Finally, considering the role that offspring might play as alloparents I studied families housed concurrently with two successive litters to investigate the effects of living with parents and older/younger siblings on offspring physical and behavioral development (Chapter 3). Mothers housed with only their mates and older offspring had lower body mass compared to mothers that raised overlapping litters. The presence of younger siblings decreased body mass of their older siblings and increased anxiety-like behavior and responsiveness to unfamiliar pups.

Consistent with previous studies (Nguyen, Zhao and Saltzman 2020; Shaikh, Asif, and Saltzman, unpublished data), mothers and sexually naïve females displayed less interest in unfamiliar pups compared to fathers and sexually naïve males (Chapters 1 and 3), suggesting that females may be constrained by their higher costs of providing maternal care in mammals. Indeed, mothers mated to younger and, possibly, higher-quality males nursed their pups more frequently than those mated to older males (Chapter 2), and maternal body mass was affected by the number of litters present while paternal body mass was not (Chapter 3). Fathers' behavior, on the other hand, was unaffected by their age (Chapter 2) or the number of litters present (Chapter 3). In our laboratory setting, the development of pups from birth to weaning age was not influenced by paternal age or the presence of older siblings (Chapters 2 and 3). On the other hand, in older offspring, only males, but not females, living with and without their younger siblings differed in their willingness to interact with an unfamiliar pup.

Altogether, my results support Trivers' (1972) parental investment theory that animals should invest in young based on the benefits of providing care relative to the costs of care. Mammalian females inherently have a higher cost of parental care than males due to necessity of pregnancy and lactation, which may explain why female California mice were less interested in unfamiliar pups compared to males. In contrast, male California mice were able to maintain levels of care in spite of age or the number of litters present, likely because the costs of providing additional care for them are low. My dissertation furthers our understanding of the role of family dynamics in caregiving behavior in a biparental mammal, revealing new avenues of study for exploring the determinants of parental and alloparental care. For example, we can examine the effects of environmental stressors, such as presence of predators or decrease in food sources, on how individual members of a family weigh the costs and benefits of providing care. Considering social and intrinsic factors on parental care may especially be informative for captive breeding programs for the conservation of endangered species.

References

Nguyen, C. T. Y., Zhao, M., & Saltzman, W. (2020). Effects of sex and age on parental motivation in adult virgin California mice. *Behavioural Processes*, *178*(June), 104185. https://doi.org/10.1016/j.beproc.2020.104185

Trivers, R. L. (1972). Parental Investment and Sexual Selection. In R. L. Trivers (Ed.), *Sexual Selection and the Descent of Man* (pp. 136–179). Routledge. https://doi.org/10.4324/9781315129266-7