Influences of Affiliative Behavior in a Socially Monogamous Mammal

by

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Dedication

To my dad, (Dr.) Brad Davidson, who has always supported me and my scientific endeavors, since I was a young child. From the humble beginnings of my rock collecting to the many cicada skins we brought home in Tupperware to the toad we watched grow from a tadpole, you have always fostered my curiosity and wonder for the world. I owe this achievement to the evenings you spent reading aloud to me and Brian, your answers to my hysteric calls about Mastering Chemistry homework, the many guinea pig memes you've shared over the years, and your constant belief that I could accomplish anything. I love you.

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"A team [doesn't] mean anything if you [can't] depend on them. That's both a big and a small thing. Knowing that there are people who will never abandon you."

— Fredrik Backman, *Beartown*

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Abstract

All animals engage in aspects of social behavior at some time in their lives, whether through mating, territory defense, or more complex sustained cooperative behaviors. Therefore, the study of social behavior allows researchers to understand the large variability in the affective valence, length, and biological relevance of interactions among dyads or groups of conspecifics. This dissertation examines several aspects of social behavior (such as space use metrics and the number and strength of social contacts) across populations of a socially monogamous mammal, the prairie vole (*Microtus ochrogaster*). Throughout this research, I aim to understand and clarify the external and internal influences of among- and within-individual variation in close social contact behavior.

Traditional methods of recording social behavior in small mammals, including live trapping and radio-frequency identification (RFID), are unable to track direct interactions or the day-to-day temporal dynamics of social relationships. Therefore, in Chapter 2, I first developed a novel biologging method to improve the temporal resolution of social behavior data collected in field settings with populations of small mammals. I compared the social behavior of free-living prairie voles as recorded by proximity loggers that detect direct interactions, live trapping, and RFID. I found that the use of proximity loggers for collecting social contact data increased the resolution of data (measured as the number of observations of an individual, social contacts recorded, and the strength of those social contacts) beyond that collected through traditional methods. In addition, I found that the proximity loggers recorded information consistent with

patterns of spatial and temporal overlap among voles, as measured by live trapping and a passive monitoring technique. I conclude that the accessibility of proximity loggers for studies of behavior in small mammals offers a promising method for improved understanding of their social behavior and its dynamic nature.

In Chapter 3, I used live trapping and a passive monitoring technique to investigate influences on social behavior in prairie voles. I compared the non-mutually exclusive hypotheses that aspects of the social environment drive social affiliation among individuals, that abiotic aspects of the physical environment drive social affiliation among individuals, and that individual traits of each vole drive their social behavior. I found that aspects of the social environment—specifically, adult sex ratio and adult population density—most reliably predicted metrics of social behavior for my study populations of prairie voles. My results indicate that aspects of demography and sociality are most influential on patterns of close social contact in this species, rather than seasonal factors. These findings add clarity to our understanding of prairie vole social behavior and help explain the behavioral variation that has been observed among populations of prairie voles for decades, by directly comparing the hypothesized influences of close social contact behavior.

Finally, in Chapter 4, I explored the effects of laboratory breeding on outcomes of field studies, during which laboratory-bred animals are released into semi-natural enclosures. Despite finding quantifiable differences among the groups of founding voles used to populate the enclosures for our studies, I found only weak evidence for differences in their natural behavior. Instead, my findings are consistent with environmental influences on social behavior. These results demonstrate that, in contrast to laboratory studies of inbred animal strains, differences in laboratory breeding colonies do not significantly alter the outcomes of enclosure studies.

Chapter 1 Introduction

1.1 Background

1.1.1 Evolution of monogamy in mammals

In behavioral ecology, a mating systems perspective allows the study of behavior on a population or species scale, tying together the influences of environmental challenges and individual fitness constraints on broad patterns of behavior and decision-making (reviewed in Rubenstein & Wrangham, 1986). Mating systems research began mainly with considerations of polygyny, in which one male mates with—and perhaps monopolizes access to—many females. Comparative work then introduced discussions of other mating systems (i.e., polyandry, polygynandry, and monogamy) and the socioecological conditions that would favor them (e.g., Orians, 1969).

Monogamy was initially defined via mating exclusivity between two opposite-sex partners, often co-occurring with behaviors such as shared territory defense, biparental care, and spatial overlap, which were less challenging than mating exclusivity to measure (Kleiman, 1977; Wittenberger & Tilson, 1980). Therefore, exclusive mating is now a signifier assigned to the more specific term, "genetic monogamy," and researchers refer more frequently to "social monogamy," which is characterized by behavioral affiliation for the duration of at least one breeding season, even in the absence of sexual fidelity (Kleiman, 1977). I will hereafter refer to social monogamy as "monogamy," unless specified otherwise.

Monogamy is a relatively rare mating system in mammals, with approximately 3–9% of species demonstrating the prerequisite pair bonding, parental behavior, communal living,

reduced sexual dimorphism, and shared patterns of physiology and neuroendocrinology that characterize the phenomenon (Carter & Perkeybile, 2018; Clutton-Brock, 1989; Kleiman, 1977; Lukas & Clutton-Brock, 2013). Due to the nature of mammalian reproduction and internal fertilization, which inherently requires heavy female investment in offspring during gestation and lactation, males are generally expected to seek as many mating opportunities as possible, as opposed to defending or raising offspring with a single mate (Bateman, 1948). Following this logic, a mating system requiring limited opposite-sex social interaction and no male parental care would be fitness-enhancing for males (Trivers, 1972). Given the significant theoretical costs of mating and associating with one select female, ethologists, evolutionary biologists, and behavioral ecologists have dedicated a wealth of research to understanding the evolution of monogamy in mammals.

Current hypotheses on the evolution of monogamy focus on the ultimate and proximate mechanisms of a transition from a solitary or promiscuous system to a monogamous one (Carter & Perkeybile, 2018; Kleiman, 1977; Lukas & Clutton-Brock, 2013; but see Agnani et al., 2018). For example, researchers have suggested that monogamy has evolved in mammals 1) because male (paternal) care is required for offspring survival (Møller & Cuervo, 2000; Silver et al., 1985) or increases offspring quality (Weatherhead & Robertson, 1979); 2) as a mechanism for infanticide avoidance (Opie et al., 2013); 3) due to scattered spatial distribution of resources and female territories, such that males are unable to defend multiple mates simultaneously (Emlen & Oring, 1977; Lukas & Clutton-Brock, 2013; Shuster & Wade, 2003); and 4) as a correlated by-product of selection on maternal care (Arnqvist & Kirkpatrick, 2005; Halliday & Arnold, 1987; Forstmeier et al., 2014). These hypotheses are not necessarily mutually exclusive, but they have generally been compared with the intention to narrow down or rank the most reliable

explanations for the evolution of monogamy. For example, studies have found that the benefits of male care are secondary to the initial evolution of monogamy.

Although there has been some support for the importance of infanticide (Borries, Savini, & Koenig, 2011; Opie et al., 2013) and distribution of female home ranges (Dunbar, 2022; Hoffmann et al., 2019; Tecot et al., 2015) as contributors to the evolution of social monogamy, especially for primates, many studies have failed to find support for any single hypothesis (Dobson et al., 2010; Fernandez-Duque, 2016; Huck et al., 2020; Reichard, 2003; Sinervo et al., 2020). Recently, Huck, DiFiore, and Fernandez-Duque (2020) began to question the basic assumption that different species and evolutionary lineages share the same explanation for the evolution of monogamy. Indeed, given that transitions to monogamy and establishment of strong affiliative behavior have independently occurred many times in mammals, this assumption may be unjustified (Lukas & Clutton-Brock, 2013; Fischer et al., 2019; Schradin, 2017). This line of inquiry remains a hot topic in evolution and behavior.

1.1.2 Pair bonding

While the overarching mystery regarding the initial evolution of monogamy remains open for further investigation, the formation of strong social bonds between opposite-sex social partners is a proposed mechanism for the *maintenance* of monogamous systems (Fletcher et al., 2015; Gavrilets, 2012; Quinlan, 2008). Behavioral endocrinology and neuroscience work has emphasized the centrality and consistency of the social pair bond within the monogamous mating system (e.g., Carter & Perkeybile, 2018).

When feasible, the presence of a pair bond is measured using the partner preference test (PPT), in which the subject animal is allowed to free roam and spend time either alone, with a social partner (previously bonded through mating and/or cohabitation) or with an unknown

conspecific (Beery, 2021; Carp et al., 2016; Williams et al., 1992). Pair bonding is indicated when the subject spends significantly more time near the established (i.e., bonded) social partner, which often includes huddling and close contact behavior. When this strong social preference is formed, bonded partners also exhibit aggression toward other individuals (Carter & Perkeybile, 2018), as recorded during experimental dyadic encounters in the laboratory (Bowler et al., 2002; Gavish et al., 1983) and playback experiments in the field (Garcia de la Chica et al., 2021). Pair bonding often co-occurs with cohabitation, territory defense, and biparental care (Kleinman, 1977; reviewed in Bales et al., 2021), but the degree of shared space use and social cohesion between pairs may vary, especially with the degree to which social monogamy is obligate as opposed to facultative (e.g., McClanahan et al., 2020).

As the measurable behavioral aspects of a pair bond develop, changes in the brain and body of a bonded animal also occur. Both the formation and maintenance of a pair bond are highly influenced by the peptide hormones oxytocin and vasopressin (Carter, 1998; Johnson & Young, 2014; Young et al., 1998, 2001; Young & Wang, 2004; reviewed in Walum & Young, 2018), although a recent study has demonstrated that oxytocin receptors are not *necessary* for the development of a pair bond, at least in prairie voles (Berendzen et al., 2023). In fact, research indicates that mechanisms of pair bond formation in the brain are complex and multifaceted interactions in the so-called social decision-making network of the brain. This network is comprised of the social behavior network (Goodson, 2005; Newman, 1999) and the mesolimbic reward system, which involve sex steroids, neuropeptide hormones, and dopaminergic connections (O'Connell & Hofmann, 2012). In addition, the hypothalamic-pituitary-adrenocortical (HPA) axis may affect pair bond formation and maintenance (DeVries, 2002). Bond disruption or separation of a bonded pair causes stress coping and depressive behaviors

(McNeal et al., 2014), along with measurable dysregulation in the HPA axis (Bosch et al., 2009; McNeal et al., 2014), dopamine (Liberwirth & Wang, 2016), endogenous opioids (Bales & Rogers, 2022), oxytocin (Bales & Rogers, 2022; Liberwirth & Wang, 2016), and vasopressin (Bales & Rogers, 2022) systems. The ties between pair bond–related behaviors and physiology may enforce and maintain the monogamous system through socially motivated behavior, as individuals find affiliative behavior rewarding and separation from a bonded partner aversive (reviewed in Resendez & Aragona, 2013).

Ultimately, the initial evolution and maintenance of strong, physiologically enforced bonds between opposite-sex social partners are hypothesized to have allowed female mammals to mitigate the costs of their high reproductive investment through food provisioning and biparental care from their mate (Fletcher et al., 2015; Kleiman, 1977). While provisioning a female mate may also benefit the male through increased offspring survival, decreased interbirth interval, and ensured paternity (via mate-guarding or gaining mate fidelity) (Gavrilets, 2012; Quinlan, 2008), he may incur high costs by vastly limiting the number of offspring he can produce (Bateman, 1948; Shuster & Wade, 2003). This system inherently creates a fitness trade-off for males, who can allocate their energy and resources to care for a single female and their offspring *or* seek a maximum number of reproductive opportunities with multiple mates.

1.1.3 Behavioral plasticity within social systems

In a world with a lack of reliable environmental cues, males may "hedge their bets" by investing in one behavioral option at random (Cohen, 1966; Philippi & Seger, 1989; Seger & Brockmann, 1987). However, when faced with a fitness challenge or rapid environmental change, animals may respond adaptively by adjusting their behavior—a phenomenon known as behavioral plasticity (Clutton-Brock, 1989; Wcislo, 1989; West-Eberhard, 1989). One well-

known example of this is the propensity of birds to alter the pitch of their calls due to anthropogenic noise pollution (reviewed in Ortega, 2012), but animals may also adjust social, mating, or parental care behavior due to changes in the social environment (Clutton-Brock, 1989).

Social variables such as local population density, familiarity with conspecifics, population sex ratio, and the behavior of group members make up the most dynamic aspects of an animal's environment (Clutton-Brock, 1989; Taborsky & Oliveira, 2012). One individual's behavior may affect others at the individual, local, or population levels in complex and nuanced ways, so the ability to detect available social information and modify behavior accordingly may allow animals to identify social challenges, avoid costly conflicts, and navigate their environment, to maximize fitness (Ghalambor et al., 2010; Taborsky & Oliveira, 2012).

These concepts may be particularly relevant to mating decisions because changes in social variables can directly influence fitness trade-offs. Mathematical models have confirmed that, for males, the fitness benefits and costs associated with seeking extra-pair copulations are dynamic and depend on the behavior of conspecifics in the population (Rice et al., 2018). Therefore, fine-tuning social and mating behavior to the social environment, via social cues that predict optimal behavior under the current conditions, should especially benefit males in monogamous systems when making mating decisions (e.g., the choice to either mate exclusively with a social partner or seek additional mating opportunities). These mating decisions are complex, nuanced, and result in important fitness consequences for individuals (Rice et al., 2018). As such, monogamous males with a higher propensity to perceive, process, and/or react to social cues should be more effective at optimizing reproduction than their peers, and individual

sources of variability in body condition or decision making should result in a fitness differential between males (Shuster & Wade, 2003; Trivers, 1972).

1.1.4 Behavioral variation in laboratory-bred animals

Many of the aforementioned studies used laboratory-bred animals to investigate the behavior, physiology, and neuroscience of social behavior. The control and standardization of variables such as genetics, environmental conditions, social interactions, and experimental methods are essential aspects of studying causality and directionality (reviewed in Olsson et al., 2003). Even in studies of animals in outdoor or semi-natural conditions, breeding wild-caught animals in the laboratory allows researchers to develop a consistent, reliable, and accessible population of research subjects. However, research has suggested that behavioral differences are more common than expected, both within (Loos et al., 2015) and across (Loos et al., 2014) laboratory populations and strains of animals, whether inbred (e.g., Bolivar et al., 2007) or outbred (e.g., deBoer et al., 2003). Such divergences in behavior are found in laboratory populations of many common laboratory model species, including insects (Huettel, 1976), zebrafish (*Danio rerio*; Audira et al., 2020; van den Bos et al., 2020; Vignet et al., 2013), mice (*Mus musculus*; e.g., Bolivar et al., 2007; An et al., 2011), and rats (*Rattus norvegicus*; e.g., Clemens et al., 2014; deBoer et al., 2003). These differences may involve anxiety-related behaviors (e.g., An et al., 2011), affiliative or antagonistic social behaviors (e.g., Faure et al., 2017), and stress coping behaviors (e.g., Cabib et al., 1990; Nosek et al., 2008).

Most strikingly, even when populations of isogenic (inbred) animals are kept under controlled conditions, such as in automated home cages, there are significant *within-strain* behavioral differences (Loos et al., 2015). There are also differences between laboratory populations of the same (inbred or outbred) genetic line, established from separate breeding

colonies. Well-studied examples of this include the skewed distributions of two Pavlovian conditioned approach responses (sign- and goal-tracking) in Sprague-Dawley (Fitzpatrick et al., 2013) and Long-Evans (Khoo et al., 2022) rats from different stocks, as well as physiological differences found in Wistar rats (Takahashi et al., 2021), DBA/2 mice (Park et al., 2021), and ICR mice (Yoon et al., 2018) from different stocks.

Studies on wild populations of animals have also found geographical differences in neophobia (e.g., Mitchell et al., 1977), sexual dimorphism (e.g., Roberts et al., 1998), and reproductive behavior (e.g., Solomon et al., 2018). However, few studies have explored the effects of laboratory breeding on wild-caught animals. Those few studies (e.g., Ophir et al., 2007) provide a springboard for the comparison of social behavior using multiple populations of laboratory-bred animals released into semi-natural enclosures.

1.1.5 Rationale for this dissertation

In this dissertation, I demonstrate proof of concept for a method to quantify and record social behavior in small mammals, which will allow the exploration of questions that have been previously impossible to answer with existing methods. I then compare existing, non-mutually exclusive hypotheses for the evolution and ontogeny of affiliative behavior, measured as close social contact, using a monogamous mammal model. This work clarifies the observation of pair-bonding and group-living behavior in this study system, setting up future experimental studies to understand the causal relationships between the social environment and the spectrum of affiliative behavior. Finally, I explore the potential for behavioral differences between populations of prairie voles bred at different laboratories, to understand whether and/or how the use of animals from separate colonies may affect the results of behavioral studies conducted in semi-natural enclosures, which have been popular for decades.

1.2 Study species

Prairie voles (*Microtus ochrogaster*) are arvicoline rodents known for their socially

monogamous mating system and commonly used as a model species for the behavior,

neuroscience, and physiology of pair bonds. Beginning in the 1980s, extensive field and

laboratory work with prairie voles has been essential to defining pair bonds (Carter & Getz,

1993), differentiating social monogamy from genetic monogamy (Getz et al., 1981), and

identifying the neurobiological mechanisms involved in social bonding (Carter et al., 1992;

Carter & Getz 1993; Insel, 1992; Insel & Shapiro, 1992; Insel et al., 1998; Young et al., 1998).

Monogamy in prairie voles is associated with selective benefits, including increased pup

survival (Getz et al., 1992), accelerated pup development (Wang & Novak, 1992), and social

buffering against physiological stress (Carter, 1998). Accordingly, opposite-sex pairs of prairie

voles readily form pair bonds, defend shared territories, participate in biparental care, and even

show cooperative breeding behaviors (i.e., offspring care by extended family or siblings, rather

than parents) at times (Getz et al., 1981, 1993). However, there is considerable variation in the

tendency for individuals to form a pair bond and invest in a single bonded social partner, as

opposed to mating with multiple individuals opportunistically (Getz et al., 1993; Ophir et al.,

2008; Solomon et al., 2009). This variation has been recorded most exhaustively in males, who

demonstrate multiple behavioral options (hereafter, "tactics"). At the two behavioral extremes,

"resident" males form pair bonds, mating exclusively and investing in parental care with one

social partner, while "wandering" males forgo pair bonding in favor of purely opportunistic,

promiscuous mating behavior (Getz et al., 1993; Solomon & Jacquot, 2002). Recently,

researchers have also identified and defined an intermediate tactic: "roving" males form pair

bonds and participate in parental care duties, while simultaneously mating opportunistically with

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extra-pair females (Rice et al., 2018). Individuals can switch between tactics during their lifetime (McGuire & Getz, 2010), and the fitness benefits of one tactic over another are dynamic, depending on the distribution of tactics throughout the population (Rice et al., 2018; Shuster et al., 2019). These tactics have traditionally been defined discretely; however, the introduction of the roving phenotype suggests that this spectrum of mating behavior is continuous and flexible (Rice et al., 2018). Therefore, it should pay for males to attune to environmental information about the mating tactics that others have adopted and adjust their behavior to match the opportunities available in the population (reviewed in Madrid et al., 2020).

Much of this work has been done in a laboratory setting, where the environment is highly controlled. Existing field studies have used live trapping and mark-recapture methods to identify social pairs through spatial and temporal overlap, which estimates broad patterns of social contact within a population. However, there is a lack of high-resolution information about the affiliative behavior of prairie voles in their natural habitat, where many factors may affect mating decisions. It remains unclear how much time pairs spend together, how they adjust parental care behavior, and how many individuals they interact with regularly in a natural setting. Although there is a consensus that extra-pair mating is common in prairie voles (Ophir et al., 2008; Solomon et al., 2009), the details of these decisions and interactions have not been recorded in real time in an ecologically relevant environment.

1.3 Study site and subjects

I collected all behavioral data and demographics information for Chapters 2 and 3 of this dissertation at the Miami University Ecology Research Center (ERC) in Oxford, Ohio (39° 53'N, 84° 73'W), over 36 months (Jun 2019–Jun 2022). This site contains 16 semi-natural, outdoor enclosures for small mammal research, surrounded by 20-gauge galvanized steel walls which

extend 75 cm aboveground and 45 cm belowground. Each enclosure is 0.1 ha (32 m x 32 m), with an electric wire running along the top of the walls to exclude terrestrial predators from the study areas. Enclosures consisted of prairie habitat, including a mix of goldenrod (*Solidago* spp.), clover (*Trifolium* spp.), timothy (*Phleum* spp.), ryegrass (*Elymus* spp.), fescue (*Festuca* spp.), bluegrass (*Poa pratensis*), and ragweed (*Ambrosia* spp.) (Solomon et al., 2009).

Populations were established in four of the semi-natural enclosures, beginning in June 2019, when 48 adult (82 - 155 days old), laboratory-bred prairie voles (originally descended from wild-caught voles in Illinois) were evenly distributed between the enclosures at a medium density natural population of 120 voles/ha (Getz et al., 1993, 2001) and equal sex ratio. An additional 28 adult (77 – 259 days old), laboratory-bred descendants of wild Illinois voles were released into the enclosures in November 2019 to reduce inbreeding and increase population density. Animals were identified with passive integrated transponder (PIT) tags (Biomark: Boise, Idaho; 12mm HPT tags) containing unique numerical IDs, which were implanted under the skin above the shoulder blades. All populations were maintained continuously in the outdoor enclosures for the course of the study, from June 2019 to June 2022. From March 8, 2020, to July 1, 2020, we did not collect data due to restrictions on research and travel from the COVID-19 pandemic.

Over the course of these three years, I collected demographic, social, and physiological data on 650 unique individuals (280 males; 329 females; 41 sex not recorded), allowing me to address the research questions in this dissertation. Animals without a recorded sex were caught a single time as juveniles and released without handling; they were never captured again (due to mortality). In addition, I used data collected by Anne Sabol during May – August 2017 and Mengxiao Zhang during May – August 2023 for Chapter 4. These data were also collected at the

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Miami University ERC, in the same enclosures, using similar methods to those briefly described above. Find the full details of these additional data in **Chapter 4**.

1.4 Research questions and chapters

In this dissertation, I sought to understand the influences of affiliative behavior in prairie voles. Past research has established that there is consistent between- and within-individual variation in affiliative behavior of prairie voles (Getz et al., 1993; McGuire & Getz, 2010; Solomon & Jacquot, 2002), but definitive evidence on the factors that drive affiliation of pairs, groups, and looser social contacts remains mixed. Similarly, there are benefits and costs associated with maintaining close social relationships (Krause & Ruxton, 2002) but the details of these may vary. I address these questions in the following three chapters of my dissertation.

In **Chapter 2**, my objective was to identify and validate a reliable method to collect fine-scale data on social affiliation and social contact between individuals with unprecedentedly high resolution. I tested a prototype of an animal-borne proximity logger, which records contacts between marked animals within 5–6 cm of each other (MD, unpublished data), and compared this method with two previously validated methods of measuring social behavior in wild rodents. These methods, live trapping and radio-frequency identification (RFID), rely exclusively on spatial and temporal overlap. I compared the resolution and patterns of social contact data, recorded as the co-occurrence of animals at the same trap location; the presence of two animals at the same RFID antenna, using a Gaussian mixture model (GMM) to group detections of animals into probable "grouping events"; and direct contact data from proximity loggers ("ProxLogs").

In **Chapter 3**, I determined the factors that most reliably predict affiliative behavior, from social variables, abiotic environmental variables, and individual traits. I compiled data over

three years of social contacts recorded from live trapping and RFID data, which I used to create a time-ordered social network. I aggregated the time-ordered data into six seasonal social networks, splitting the data into late autumn–early spring and late spring–early autumn based on previous research, and extracted summary statistics to measure the number of unique social connections for each individual (i.e., unweighted degree) and the strength of those connections (i.e., degree weight) during each season. Using *a priori* predictions, I compared a set of models which represented the non-mutually exclusive hypotheses that social factors, abiotic factors, or individual factors most reliably predicted close social contact behavior.

In **Chapter 4**, I explored possible behavioral divergences between sets of voles bred in different laboratory colonies at three different universities. I used live trapping and RFID data collected over the course of three unrelated studies, which founded semi-natural populations of prairie voles from animals acquired from the Nancy Solomon-Brian Keane Laboratory (Miami University; Oxford, OH), the Angela Grippo Laboratory (Northern Illinois University; DeKalb, IL), and the James Burkett Laboratory (University of Toledo; Toledo, OH). I quantified differences in body weight and age for the individuals acquired from each laboratory prior to introduction into the semi-natural enclosures using Kruskall-Wallis tests with post-hoc Dunn Tests adjusted with the Holm method. I compared survival to the end of the study period, using a binomial regression to compare populations of founding voles. I then compared home range area and degree of home range overlap among individuals from each breeding colony, as a means to explore any differences in space use or mating tactic. Finally, I compared the number of unique social connections (i.e., unweighted degree) and the strength of those connections (i.e., degree weight) to determine whether there were structural differences in social interactions among the groups of founding voles acquired from each laboratory.

Although I am the first author for each of these chapters, all of this work has been a shared effort with my advisor, Dr. Ben Dantzer, and my collaborators Dr. Nancy Solomon and Dr. Brian Keane. Therefore, I will hereafter refer to our collaborative work using the pronouns "we/our/ours" in the remaining chapters.

1.5 Significance of research

This dissertation adds to the significant body of knowledge about prairie vole behavior, pair bonding, and the maintenance of monogamy. To our knowledge, this is the first study using proximity loggers on small, semi-fossorial mammals in a naturalistic setting. Our research indicates that advancements in proximity logger technology enable researchers to understand fine-scale patterns of interaction by collecting continuous, 24-hour data in areas where it has been previously impossible to record behavior. In addition to the validation of a novel method for recording social interactions, this dissertation clarifies the factors contributing to affiliative behavior and demonstrates a lack of evidence for divergences in behavior based on the use of founding voles from different breeding colonies. These are some of the first studies to directly compare hypotheses regarding seasonal variation in social behavior or quantify demographic and social differences in populations established from multiple breeding colonies. Therefore, the work in this dissertation is essential to advancing field methods, as well as setting up future, causal studies on pair bonding and monogamy in natural settings.

1.6 References

Agnani, P., Kauffmann, C., Hayes, L. D., & Schradin, C. (2018). Intra-specific variation in social organization of Strepsirrhines. *American Journal of Primatology, 80*(5), e22758. https://doi.org/10.1002/ajp.22758.

An, X. L., Zou, J. X., Wu, R. Y., Yang, Y., Tai, F. D., Zeng, S. Y., Zhang, X., Liu, E., & Broders, H. (2011). Strain and sex differences in anxiety-like and social behaviors in

C57BL/6J and BALB/cJ mice. *Experimental animals*, *60*(2), 111-123. https://doi.org/10.1538/expanim.60.111.

Arnqvist, G., & Kirkpatrick, M. (2005). The evolution of infidelity in socially monogamous passerines: The strength of direct and indirect selection on extrapair copulation behavior in females. *The American Naturalist, 165 Suppl 5*, S26-S37. https://doi.org/10.1086/429350.

Audira, G., Siregar, P., Strungaru, S. A., Huang, J. C., & Hsiao, C. D. (2020). Which zebrafish strains are more suitable to perform behavioral studies? A comprehensive comparison by phenomic approach. *Biology*, *9*(8), 200. https://doi.org/10.3390/biology9080200.

Bales, K. L., Ardekani, C. S., Baxter, A., Karaskiewicz, C. L., Kuske, J. X., Lau, A. R., Savidge, L. E., Sayler, K. R., & Witczak, L. R. (2021). What is a pair bond?. *Hormones and Behavior, 136*, 105062. https://doi.org/10.1016/j.yhbeh.2021.105062.

Bales, K. L., & Rogers, F. D. (2022). Interactions between the κ opioid system, corticotropin-releasing hormone and oxytocin in partner loss. *Philosophical Transactions of the Royal Society B, 377*(1858), 20210061. https://doi.org/10.1098/rstb.2021.0061.

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity, 2*, 349–368. https://doi.org/10.1038/hdy.1948.21.

Beery, A. K. (2021). Familiarity and mate preference assessment with the partner preference test. *Current Protocols, 1*, e173. https://doi.org/10.1002/cpz1.173.

Berendzen, K. M., Sharma, R., Mandujano, M. A., Wei, Y., Rogers, F. D., Simmons, T. C., Seelke, A. M. H., Bond, J. M., Larios, R., Goodwin, N. L., Sherman, M., Parthasarthy, S., Espineda, I., Knoedler, J. R., Beery, A., Bales, K. L., Shah, N. M., & Manoli D. S. (2023). Oxytocin receptor is not required for social attachment in prairie voles. *Neuron, 111*(6), P787-796.E4. https://doi.org/10.1016/j.neuron.2022.12.011.

Borries, C., Savini, T., & Koenig, A. (2011). Social monogamy and the threat of infanticide in larger mammals. *Behavioral Ecology and Sociobiology, 65*(4), 685-693. https://doi.org/10.1007/s00265-010-1070-5.

Bosch, O. J., Nair, H. P., Ahern, T. H., Neumann, I. D., & Young, L. J. (2009). The CRF system mediates increased passive stress-coping behavior following the loss of a bonded partner in a monogamous rodent. *Neuropsychopharmacology, 34*(6), 1406-1415. https://doi.org/10.1038/npp.2008.154.

Bolivar, V. J., Walters, S. R., & Phoenix, J. L. (2007). Assessing autism-like behavior in mice: variations in social interactions among inbred strains. *Behavioural Brain Research*, *176*(1), 21-26. https://doi.org/10.1016/j.bbr.2006.09.007.

Bowler, C. M., Cushing, B. S., & Carter, C. S. (2002). Social factors regulate female–female aggression and affiliation in prairie voles. *Physiology & behavior, 76*(4-5), 559-566. https://doi.org/10.1016/S0031-9384(02)00755-2.

Cabib, S., Algeri, S., Perego, C., & Puglisi-Allegra, S. (1990). Behavioral and biochemical changes monitored in two inbred strains of mice during exploration of an unfamiliar environment. *Physiology & Behavior, 47*(4), 749-753. https://doi.org/10.1016/0031-9384(90)90089-M.

Carp, S. B., Rothwell, E. S., Bourdon, A., Freeman, S. M., Ferrer, E., & Bales, K. L. (2016). Development of a partner preference test that differentiates between established pair bonds and other relationships in socially monogamous titi monkeys (*Callicebus cupreus*). *American Journal of Primatology, 78*(3), 326-339. https://doi.org/10.1002/ajp.22450.

Carter, C. S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology, 23*(8), 779-818. https://doi.org/10.1016/S0306-4530(98)00055-9.

Carter, C. S., Williams, J. R., Witt, D. M., & Insel, T. R. (1992). Oxytocin and social bonding. *Annals of the New York Academy of Sciences, 652*, 204–211. https://doi.org/10.1111/j.1749-6632.1992.tb34356.x

Carter, C. S., & Getz, L. L. (1993). Monogamy and the prairie vole. *Scientific American, 268*(6): 100-106. https://doi.org/10.1038/scientificamerican0693-100.

Carter, C. S., & Perkeybile, A. M. (2018). The monogamy paradox: what do love and sex have to do with it? *Frontiers in Ecology & Evolution, 29*, 1-6. https://doi.org/10.3389/fevo.2018.00202.

Clemens, L. E., Jansson, E. K. H., Portal, E., Riess, O., & Nguyen, H. P. (2014). A behavioral comparison of the common laboratory rat strains Lister Hooded, Lewis, Fischer 344 and Wistar in an automated homecage system. *Genes, Brain and Behavior*, *13*(3), 305-321. https://doi.org/10.1111/gbb.12093.

Clutton-Brock, T. H. (1989). Review lecture: mammalian mating systems. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 236*, 339–372. https://doi.org/10.1098/rspb.1989.0027.

Cochran, G. R., & Solomon, N. G. (2000). Effects of food supplementation on the social organization of prairie voles (Microtus ochrogaster). *Journal of Mammalogy, 81*(3), 746-757. https://doi.org/10.1644/1545-1542(2000)081%3C0746:EOFSOT%3E2.3.CO;2.

Cohen, D. (1966). Optimizing reproduction in a randomly varying environment. *Journal of Theoretical Biology, 12*(1), 119-129. https://doi.org/10.1016/0022-5193(66)90188-3.

de Boer, S. F., van der Vegt, B. J., & Koolhaas, J. M. (2003). Individual variation in aggression of feral rodent strains: a standard for the genetics of aggression and violence?. *Behavior genetics*, *33*, 485-501. https://doi.org/10.1023/A:1025766415159.

DeVries, A. C. (2002). Interaction among social environment, the hypothalamic–pituitary–adrenal axis, and behavior. *Hormones and Behavior, 41*(4), 405-413. https://doi.org/10.1006/hbeh.2002.1780.

Dobson, F. S., Way, B. M., & Baudoin, C. (2010). Spatial dynamics and the evolution of social monogamy in mammals. *Behavioral Ecology, 21*(4), 747-752. https://doi.org/10.1093/beheco/arq048.

Dunbar, R. I. M. (2022). Female dispersion is necessary, but not sufficient, for pairbonded monogamy in mammals. *Frontiers in Ecology and Evolution, 17*(10). https://doi.org/10.3389/fevo.2022.905298.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*, 215-223. https://doi.org/10.1126/science.327542.

Faure, A., Pittaras, E., Nosjean, A., Chabout, J., Cressant, A., & Granon, S. (2017). Social behaviors and acoustic vocalizations in different strains of mice. *Behavioural brain research*, *320*, 383-390. https://doi.org/10.1016/j.bbr.2016.11.003.

Fernandez-Duque, E. (2016). Social monogamy in wild owl monkeys (*Aotus azarae*) of Argentina: The potential influences of resource distribution and ranging patterns. *American Journal of Primatology, 78*(3), 355-371. https://doi.org/10.1002/ajp.22397.

Fischer, E. K., Nowicki, J. P., & O'Connell, L.A. (2019). Evolution of affiliation: Patterns of convergence from genomes to behaviour. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 374*(1777). https://doi.org/10.1098/rstb.2018.0242.

Fitzpatrick, C. J., Gopalakrishnan, S., Cogan, E. S., Yager, L. M., Meyer, P. J., Lovic, V., ... & Morrow, J. D. (2013). Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. *PloS one*, *8*(10), e75042. https://doi.org/10.1371/journal.pone.0075042.

Fletcher, G. J. O., Simpson, J. A., Campbell, L., & Overall, N. C. (2015). Pair-bonding, romantic love, and evolution: The curious case of *Homo sapiens*. P*erspectives on Psychological Science, 10*(1), 20-36. https://doi.org/10.1177/1745691614561683.

Forstmeier, W., Nakagawa, S., Griffith, S. C., & Kempenaers, B. (2014). Female extra-pair mating: Adaptation or genetic constraint? *Trends in Ecology and Evolution, 29*(8), 456-464. https://doi.org/10.1016/j.tree.2014.05.005.

Franks, D. W., Ruxton, G. D., & James, R. Sampling animal association networks with the gambit of the group. *Behavioral Ecology & Sociobiology 64*, 493–503 (2010). https://doi.org/10.1007/s00265-009-0865-8.

Garcia de la Chica, A., Wood, D. B., Rotundo, M., & Fernandez-Duque, E. (2021). Responses of a pair-living, sexually monogamous primate to the simulated presence of solitary individuals: A field playback experiment. *Ethology*, *127*(11), 1002-1018. https://doi.org/10.1111/eth.13222.

Gavish, L., Carter, C. S., & Getz, L. L. (1983). Male-female interactions in prairie voles. Animal Behaviour, 31(2), 511-517. https://doi.org/10.1016/S0003-3472(83)80073-6.

Gavrilets, S. (2012). Human origins and the transition from promiscuity to pair-bonding. *PNAS, 109*(25), 9923-9928. https://doi.org/10.1073/pnas.1200717109.

Getz, L. L., Carter, C. S., & Gavish, L. (1981). The mating system of the prairie vole, *Microtus ochrogaster*: Field and laboratory evidence for pair-bonding. *Behavioral Ecology and Sociobiology*, 189-194. https://doi.org/10.1007/BF00299829.

Getz, L. L., Larson, C. M., & Lindstrom, K. A. (1992). Blarina brevicauda as a predator on nestling voles. *Journal of Mammalogy, 73*(3), 591-596. https://doi.org/10.2307/1382030.

Getz, L. L., McGuire, B., Pizzuto, T., Hofmann, J. E., & Frase, B. (1993). Social organization of the prairie vole (*Microtus ochrogaster*). *Journal of Mammalogy, 74*(1), 44–58. https://doi.org/10.2307/1381904.

Getz, L. L., Hofmann, J. E., McGuire, B., & Dolan III, T. W. (2001). Twenty-five years of population fluctuations of *Microtus ochrogaster* and *M. pennsylvanicus* in three habitats in east-central Illinois. *Journal of Mammalogy, 82*, 22-34. http://dx.doi.org/10.1093/jmammal/82.1.22.

Ghalambor, C.K., Angeloni, L.M., & Carroll, S.P. (2010). Behavior as phenotypic plasticity. In: *Evolutionary Behavioral Ecology* (Eds. Westneat D.F., Fox C.W.), pp 90-107. Oxford University Press, New York, NY.

Goodson, J. L. (2005). The vertebrate social behavior network: evolutionary themes and variations. *Hormones and Behavior, 48*(1), 11-22. https://doi.org/10.1016/j.yhbeh.2005.02.003.

Grange, W. B. (1932). The Pelages and Color Changes of the Snowshoe Hare, Lepus americanus phaeonotus, Allen. *Journal of Mammalogy, 13*(2), 99–116. https://doi.org/10.2307/1374046.

Halliday, T., & Arnold, S. J. (1987). Multiple mating by females – A perspective from quantitative genetics. *Animal Behaviour 35*(3), 939-941. https://doi.org/10.1016/S0003-3472(87)80138-0.

Hoffmann, S., Bennett, N. C., van Vuuren, B. J., & Lutermann, H. (2019). Space use and the evolution of social monogamy in eastern rock sengis. *Ethology, 126*(4), 393-402. https://doi.org/10.1111/eth.12983.

Huck, M., Di Fiore, A., & Fernandez-Duque, E. (2020). Of apples and oranges? The evolution of "mongamy" in non-human primates. *Frontier in Ecology and Evolution, 10*(7). https://doi.org/10.3389/fevo.2019.00472.

Huettel, M. D. (1976). Monitoring the quality of laboratory-reared insects: a biological and behavioral perspective. *Environmental Entomology*, *5*(5), 807-814. https://doi.org/10.1093/ee/5.5.807.

Insel, T. R. (1992). Oxytocin—a neuropeptide for affiliation: evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology*, 17(1), 3-35. https://doi.org/10.1016/0306-4530(92)90073-G.

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences, 89*(13), 5981-5985.

Insel, T. R., Winslow, J. T., Wang, Z., & Young, L. J. (1998). Oxytocin, vasopressin, and the neuroendocrine basis of pair bond formation. *Vasopressin and oxytocin: Molecular, cellular, and clinical advances*, 215-224.

Johnson, Z. V., & Young, L. J. (2014). Neurobiological mechanisms of social attachment and pair bonding. *Current Opinion in Behavioral Sciences, 3*, 38-22. https://doi.org/10.1016/j.cobeha.2015.01.009.

Kleiman, D. (1977). Monogamy in Mammals. *The Quarterly Review of Biology, 52*(1): 39-69. https://doi.org/10.1086/409721.

Khoo, S. Y. S., Uhrig, A., Samaha, A. N., & Chaudhri, N. (2022). Does vendor breeding colony influence sign-and goal-tracking in Pavlovian conditioned approach? A preregistered empirical replication. *Neuroanatomy and Behaviour, 4*(1), e46. https://doi.org/10.35430/nab.2022.e46.

Krause, J., & Ruxton, G. (2002). *Living in Groups.* Oxford University Press, USA. ISBN: 0198508174.

Loos, M., Koopmans, B., Aarts, E., Maroteaux, G., Van Der Sluis, S., Neuro-BSIK Mouse Phenomics Consortium, Verhage, M., & Smit, A. B. (2014). Sheltering behavior and locomotor activity in 11 genetically diverse common inbred mouse strains using home-cage monitoring. *PLoS One, 9*(9), e108563. https://doi.org/10.1371/journal.pone.0108563.

Loos, M., Koopmans, B., Aarts, E., Maroteaux, G., van der Sluis, S., Neuro-BSIK Mouse Phenomics Consortium, Verhage, M., & Smit, A. B. (2015). Within-strain variation in behavior differs consistently between common inbred strains of mice. *Mammalian genome, 26*, 348-354. https://doi.org/10.1007/s00335-015-9578-7.

Lukas, D., & Clutton-Brock, T. H. (2013). The evolution of social monogamy in mammals. *Science, 341*, 526-530. https://doi.org/10.1126/science.1238677.

Madrid, J. E., Parker, K. J., & Ophir, A. G. (2020). Variation, plasticity, and alternative mating tactics: revisiting what we know about the socially monogamous prairie vole. *Advances in the Study of Behavior, 52*, 203-242. https://doi.org/10.1016/bs.asb.2020.02.001.

McClanahan, K., Rosell, F., & Mayer, M. (2020). Minding your own business: low pair cohesion in a territorial, monogamous mammal. *Animal Behaviour, 166,* 119-128. https://doi.org/10.1016/j.anbehav.2020.05.008.

McGuire, B., & Getz, L. L. (2010). Alternative male reproductive tactics in a natural population of prairie voles *Microtus ochrogaster*. *Acta Theriologica, 55*, 261-270. https://doi.org/10.4098/j.at.0001-7051.077.2009.

McNeal, N., Scotti, M. A. L., Wardwell, J., Chandler, D. L., Bates, S. L., LaRocca, M., Trahanas, D. M., & Grippo, A. J. (2014). Disruption of social bonds induces behavioral and physiological dysregulation in male and female prairie voles. *Autonomic Neuroscience, 180*, 9-16. https://doi.org/10.1016/j.autneu.2013.10.001.

Mitchell, D., Beatty, E. T., & Cox, P. K. (1977). Behavioral differences between two populations of wild rats: implications for domestication research. *Behavioral Biology, 19*(2), 206-216. https://doi.org/10.1016/S0091-6773(77)91494-8.

Møller, A. P., & Cuervo, J. J. (2000). The evolution of paternity and paternal care in birds. *Behavioral Ecology*, *11*(5), 472-485. https://doi.org/10.1093/beheco/11.5.472.

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior a node in the mammalian social behavior network. *Annals of the New York Academy of Sciences, 877*(1), 242-257. https://doi.org/10.1111/j.1749-6632.1999.tb09271.x.

Nosek, K., Dennis, K., Andrus, B. M., Ahmadiyeh, N., Baum, A. E., Woods, L. C. S., & Redei, E. E. (2008). Context and strain-dependent behavioral response to stress. *Behavioral and Brain Functions*, *4*(1), 1-8. https://doi.org/10.1186/1744-9081-4-23.

O'Connell, L. A., & Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science, 336*(6085). 1154-1157. https://doi.org/10.1126/science.1218889.

Olsson, I. A. S., Nevison, C. M., Patterson-Kane, E. G., Sherwin, C. M., Van de Weerd, H. A., & Würbel, H. (2003). Understanding behaviour: the relevance of ethological approaches in laboratory animal science. *Applied Animal Behaviour Science*, *81*(3), 245-264. https://doi.org/10.1016/S0168-1591(02)00285-X.

Ophir, A. G., Phelps, S. M., Sorin, A. B., & Wolff, J. O. (2007). Morphological, genetic, and behavioral comparisons of two prairie vole populations in the field and laboratory. *Journal of Mammalogy*, *88*(4), 989-999.https://doi.org/10.1644/06-MAMM-A-250R.1.

Ophir, A. G., Phelps, S. M., Sorin, A. B., & Wolff, J. O. (2008). Social but not genetic monogamy is associated with greater breeding success in prairie voles. A*nimal Behaviour, 75*(3), 1143-1154. https://doi.org/10.1016/j.anbehav.2007.09.022.

Opie, C., Atkinson, Q. D., Dunbar, R. I. M., & Shultz., S. (2013). Male infanticide leads to social monogamy in primates. *PNAS, 110*, 13328-13332. https://doi.org/10.1073/pnas.1307903110.

Orians, G. H. (1969). On the evolution of mating systems in birds and mammals. *The American Naturalist, 103*(934), 589-603. https://doi.org/10.1086/282628.

Ortega, C. P. (2012). Chapter 2: Effects of noise pollution on birds: A brief review of our knowledge. *Ornithological Monographs, 74*(1), 6-22. https://doi.org/10.1525/om.2012.74.1.6.

Park, J. W., Lee, S. J., Kim, J. E., Kang, M. J., Bae, S. J., Choi, Y. J., ... & Song, H. K. (2021). Comparison of response to LPS-induced sepsis in three DBA/2 stocks derived from

different sources. *Laboratory Animal Research, 37*(1), 1-9. https://doi.org/10.1186/s42826-020-00079-5.

Philippi, T., & Seger, J. (1989). Hedging one's evolutionary bets, revisited. *Trends in Ecology & Evolution, 4*(2), 41-44. https://doi.org/10.1016/0169-5347(89)90138-9.

Psorakis, I., Voelkl, B., Garroway, C. J., Radersma, R., Aplin, L. M., Crates, R. A., ... & Sheldon, B. C. (2015). Inferring social structure from temporal data. *Behavioral Ecology and Sociobiology*, *69*, 857-866. https://doi.org/10.1007/s00265-015-1906-0.

Quinlan, R. J. (2008). Human pair-bonds: Evolutionary functions, ecological variation, and adaptive development. *Evolutionary Anthropology, 17*, 227-238. https://doi.org/10.1002/evan.20191.

Raulo, A., & Dantzer, B. (2018). Associations between glucocorticoids and sociality across a continuum of vertebrate social behavior. *Ecology and Evolution, 8*(15), 7697-7716. https://doi.org/10.1002/ece3.4059.

Reichard, U. H. (2003). Social monogamy in gibbons: the male perspective. *Monogamy: mating strategies and partnerships in birds, humans and other mammals.* Cambridge University Press, Cambridge, 190-213.

Resendez, S. L., & Aragona, B. J. (2013). Aversive motivation and the maintenance of monogamous pair bonding. *Reviews in the Neurosciences, 24*(1), 51-60. https://doi.org/10.1515/revneuro-2012-0068.

Rice, M. A., Restrepo, L. F., & Ophir, A. G. (2018). When to cheat: Modeling dynamics of paternity and promiscuity in socially monogamous prairie voles (*Microtus ochrogaster*). *Frontiers in Ecology and Evolution, 6*(141). https://doi.org/10.3389/fevo.2018.00141.

Roberts, R. L., Williams, J. R., Wang, A. K., & Carter, C. S. (1998). Cooperative breeding and monogamy in prairie voles: influence of the sire and geographical variation. *Animal Behaviour*, *55*(5), 1131-1140. https://doi.org/10.1006/anbe.1997.0659.

Rubenstein, D. I., & Wrangham, R. W. (1986). Socioecology: origins and trends. *Ecological aspects of social evolution*, 3-17.

Sabol, A. C., Solomon, N. G., & Dantzer, B. (2018). How to study socially monogamous behavior in secretive animals? Using social network analyses and automated tracking systems to study the social behavior of prairie voles. *Frontiers in Ecology and Evolution, 6*, 178. https://doi.org/10.3389/fevo.2018.00178.

Saxbe, D., & Repetti, R. L. (2010). For better or worse? Coregulation of couples' cortisol levels and mood states. *Journal of Personality and Social Psychology, 98*(1), 92. https://doi.org/10.1037/a0016959.

Schradin, C. (2017). Comparative studies need to rely both on sound natural history data and on excellent statistical analysis. *Royal Society Open Science, 4*(9), 170346. https://doi.org/10.1098/rsos.170346.

Seger, J. & Brockmann, H. J. (1987). What is bet hedging? In: *Oxford Surveys in Evolutionary Biology* (eds P.H. Harvey & L. Partridge) Oxford University Press, Oxford, pp. 182–211.

Shuster, S. M., Willen, R. M., Keane, B., & Solomon, N. G. (2019). Alternative mating tactics in socially monogamous prairie voles, *Microtus ochrogaster*. *Frontiers in Ecology and Evolution, 7*, 7. https://doi.org/10.3389/fevo.2019.00007.

Shuster, S. M., & Wade, M. J. (2003). *Mating systems and mating strategies.* Princeton University Press. ISBN 0-691-04931-9.

Silver, R., Andrews, H., & Ball, G. (1985). Parental care in an ecological perspective: A quantitative analysis of avian subfamilies. *American Zoologist, 25*, 823-840. https://doi.org/10.1093/icb/25.3.823.

Sinervo, B., Chaine, A. S., & Miles, D. B. (2020). Social games and genic selection drive mammalian mating system evolution and speciation. *The American Naturalist, 195*(2), 247-274. https://doi.org/10.1086/706810.

Solomon, N. G., & Jacquot, J. J. (2002). Characteristics of resident and wandering prairie voles, Microtus ochrogaster. *Canadian Journal of Zoology, 80*(5), 951-955. https://doi.org/10.1139/z02-053.

Solomon, N. G., Richmond, A. R., Harding, P. A., Fries, A., Jacquemin, S., Schaefer, R. L., Lucia, K.E., & Keane, B. (2009). Polymorphism at the avpr1a locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Molecular Ecology, 18*(22), 4680-4695. https://doi.org/10.1111/j.1365-294X.2009.04361.x.

Taborsky, B., & Oliveira, R.F. (2012). Social competence: an evolutionary approach. *Trends in Ecology and Evolution, 27*,679-688. https://doi.org/10.1016/j.tree.2012.09.003.

Takahashi, K., Shima, T., Soya, M., Oharomari, L. K., Okamoto, M., & Soya, H. (2021). Differences in exercise capacity and physiological responses in Wistar rats among breeders. *Experimental Animals*, *70*(4), 508-513. https://doi.org/10.1538/expanim.21-0057.

Tecot, S. R., Singletary, B., & Eadie, E. (2016), Why "monogamy" isn't good enough. *American Journal of Primatology, 78*, 340-354. https://doi.org/10.1002/ajp.22412.

Trivers, R. L. (1972). Parental investment and sexual selection. In B. G. Campbell (Ed.), *Sexual selection and the descent of man* (pp. 136–179). Routledge.

van den Bos, R., Flik, G., & Gorissen, M. (2020). Behavioral research in zebrafish (Danio rerio): strain as source of variation. In *Behavioral and Neural Genetics of Zebrafish* (pp. 245-262). Academic Press.

Vignet, C., Bégout, M. L., Péan, S., Lyphout, L., Leguay, D., & Cousin, X. (2013). Systematic screening of behavioral responses in two zebrafish strains. *Zebrafish*, *10*(3), 365-375. https://doi.org/10.1089/zeb.2013.0871.

Walum, H., & Young, L.J. (2018). The neural mechanisms and circuitry of the pair bond. *Nature Reviews Neuroscience ,19*, 643–654. https://doi.org/10.1038/s41583-018-0072-6.

Wang, Z., & Novak, M. A. (1992). Influence of the social environment on parental behavior and pup development of meadow voles (Microtus pennsylvanicus) and prairie voles (M. ochrogaster). *Journal of Comparative Psychology, 106*(2), 163. https://psycnet.apa.org/doi/10.1037/0735-7036.106.2.163.

Wcislo, W. (1989). Behavioral environments and evolutionary change. *Annual Review of Ecology and Systematics, 20*(1), 137–169. https://doi.org/10.1146/annurev.es.20.110189.001033.

West-Eberhard, M. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics, 20*(1), 249–278. https://doi.org/10.1146/annurev.es.20.110189.001341.

Weatherhead, P. J., & Robertson, R. J. (1979). Offspring quality and the polygyny threshold: "The sexy son hypothesis". *The American Naturalist, 113*, 201-208. https://doi.org/10.1086/283379.

Whitehead, H., & Dufault, S. (1999). Techniques for analyzing vertebrate social structure using identified individuals. *Adv Stud Behav*, *28*, 33-74.

Williams, J. R., Catania, K. C., & Carter, C. S. (1992). Development of partner preferences in female prairie voles (*Microtus ochrogaster*): The role of social and sexual experience. *Hormones and Behavior, 26*(3), 339-349. https://doi.org/10.1016/0018-506X(92)90004-F.

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

Yoon, W. B., Choi, H. J., Kim, J. E., Park, J. W., Kang, M. J., Bae, S. J., ... & Song, H. K. (2018). Comparison of scopolamine-induced cognitive impairment responses in three different ICR stocks. *Laboratory Animal Research*, *34*, 317-328. https://doi.org/10.5625/Iar.2018.34.4.317.

Young, L. J., Wang, Z., & Insel, T. R. (1998). Neuroendocrine bases of monogamy. *Trends in Neurosciences, 21*(2), P71-75. https://doi.org/10.1016/S0166-2236(97)01167-3.

Young, L. J., Lim, M. M., Gingrich, B., & Insel, T. R. (2001). Cellular mechanisms of social attachment. *Hormones and Behavior, 40*(2), 133-138. https://doi.org/10.1006/hbeh.2001.1691.

Young, L. J, & Wang, Z. (2004). The neurobiology of pair bonding. Nature Neuroscience, 7, 1048-1054. https://doi.org/10.1038/nn1327.

Chapter 2 Methods for Measuring Fine Scale Social Behavior in Small Mammals

2.1 Abstract

Observing animals is an important aspect of data collection for animal behavior research, but there are barriers to directly observing animals that are cryptic or live in environments which are inaccessible to humans. Biologgers, which use animal-borne devices to collect data on animals without a human researcher present, have become essential tools for gathering data on animals that cannot be directly observed, as well as reducing the effort and resources required for data collection. However, the use of biologgers has historically been size-limited, until recent efforts to miniaturize the technology opened up the possibility of using them on small animals. In this study, we packaged and deployed proximity logging devices ("ProxLogs") for use on a small, monogamous, semi-fossorial mammal, the prairie vole, as a means to collect high-resolution data on fine-scale social behavior. Animal-borne proximity logging devices record direct interactions among animals wearing the devices, when they are within ~5 cm of each other (MD, unpublished data). We found that UV-curing epoxy resin was a durable packaging material for our ProxLogs, resistant to chewing by voles and abrasions by soil. We compared the resolution of social contact data collected by ProxLogs to patterns of temporal-spatial overlap collected through two traditional methods: live trapping and radio frequency identification (RFID) detections of PIT tagged animals. We found that the ProxLogs generated significantly higher-resolution data than traditional methods, by all metrics that we measured. We also found that social contact data collected through live trapping significantly predicted the social contact

data collected by the ProxLogs. We discuss the proof of concept we have demonstrated here, as well as future possibilities and recommendations for the generalized use of proximity loggers in small animal research.

2.2 Introduction

The observation of animals is an essential feature of animal behavior research, especially when studying social behavior among conspecifics. For decades, researchers have used visual observation to quantify interactions—for example, in long-term studies on lions (*Panthera leo*, e.g., Serengeti Lion Project; Packer, 2019), cheetahs (*Acinonyx jubatus*, e.g., Serengeti Cheetah Project; Durant et al., 2007), blue monkeys (*Cercopithecus mitis stuhlmanni*; e.g., Kakamega Monkey Project; Cords, 2012), yellow baboons (*Papio cynocephalus*; e.g., Amboseli Baboon Project; Alberts & Altman, 2012), chimpanzees (*Pan troglodytes*; e.g., Chimpanzees of Gombe National Park; Wilson, 2012), and white-faced capuchins (*Cebus capucinus*; e.g., Lomas Barbudal Monkey Project; Perry et al., 2012*)*, among many others. Small mammals have primarily been observed using capture, mark, release techniques (reviewed in Sanderson, 1966), which allow animals to be recognized on sight (e.g., dye marking of Arctic ground squirrels, *Citellus undulatus*; Melchior & Iwen, 1965) and document aspects of population demography, growth rates, dispersal, and health (e.g., McGuire et al., 2006). Additionally, the use of multiple-capture traps has enabled the study of social organization and interactions among animals captured in the same trap (Getz et al., 1986).

However, some animals are difficult to directly observe, and many details about the behaviors of individuals and populations are not capturable using traditional methods of momentary or indirect observation. Thus, many important behavioral details remain unknown for cryptic or inaccessible study species (Krause et al., 2013). Techniques such as live trapping, mist

netting (e.g., Flaquer et al., 2007), and very high frequency (VHF) radio telemetry (Craighead and Craighead 1966; Amlaner & MacDonald 1980) are labor intensive for researchers and invasive for the study animals (Bosson et al., 2012; Delehanty et al., 2009; Fletcher & Boonstra, 2006; Lynn & Porter, 2008;). In addition, radio frequency identification (RFID) systems have been used to detect passive integrated transponder (PIT) tags implanted subcutaneously in freely-living prairie voles as they moved through natural grass runways (Harper & Batzli, 1996). Since the 1990s, the use of RFID technology to detect PIT tagged animals has been utilized in aquatic research to detect migration and abundance of fish (e.g., Fetherman et al., 2014), as well as feeding, flocking, and nesting patterns of birds (reviewed in Bonter & Bridge, 2011) and mammals provided with nest boxes (e.g., König et al., 2015), feeding stations (e.g., Morris, 2016), or movement corridors (e.g., Morris & MacEachern, 2010; Rehmeier et al., 2006).

More recent studies (e.g., Sabol et al., 2018, 2020, 2023) have used RFID systems to record the movement of PIT tagged animals and use patterns of spatial-temporal overlap to infer the likelihood of social interactions between prairie voles (*Microtus ochrogaster*). However, although these systems improve the spatial and temporal resolution of data beyond that of traditional live trapping methods (Sabol et al., 2018), they are unable to directly measure social interactions due to collision between the signals of multiple tags simultaneously at an antenna (Yang et al., 2010; MD personal observation). Signal collision occurs when two devices try to simultaneously transmit data and the two signals interfere with each other, causing data loss (Yang et al., 2010).

Advances in the last 20 years of biologging technology—which uses animal-borne data loggers to record information about animals in the absence of a human observer—have made it possible to study animals with cryptic behavior or living in inaccessible environments (Krause et

al., 2013). Since the early 2000s, researchers have been using proximity loggers to detect direct contacts among wild animals. Proximity loggers are animal-borne devices that use wireless signals (e.g., VHF radio waves or low-energy Bluetooth) to log contacts between individuals at close range. For example, contacts have been recorded among wild brushtail possums (*Trichosurus vulpecula*) to understand shifts in social behavior over time and their epidemiological implications (Ji et al., 2005). Similarly, proximity loggers were used to record social contacts between raccoons (*Procyon lotor*), and the researchers concluded that proximity loggers offered an improved method for assessing the social behavior of secretive or difficult-to-observe species (Prange et al., 2006). Developed in the 2010s, the Encounternet proximity logging system (Mennill et al., 2012) allowed researchers to use VHF radio telemetry receiver stations for long-term monitoring of free-living animals. This system was tested on amphibians (Meise et al., 2013) and birds (Levin et al., 2015; Snijders et al., 2014), but ultimately failed to become publicly available and widely accessible to researchers. Similarly, the automated radiotelemetry system (ARTS) may be promising for collecting high-resolution passive data on freely moving mammals, but it has not yet been validated for measuring social behavior (Wallace et al., 2021). Neither of these systems are commercially available for researcher use.

Although proximity loggers have been available since the early 2000s, their size and battery weight made them unusable for studies of small mammals until quite recently (Krause et al., 2013). The development and testing of proximity loggers for small animals is integral to furthering our understanding of animal sociality (Ryder et al., 2012). Proximity loggers have been successfully deployed on roosting greater mouse-eared bats (*Myotis myotis*), demonstrating that it is possible to develop miniaturized proximity loggers for use on small mammals (Ripperger et al., 2016). However, the packaging and attachment methods of biologgers vary

considerably. They must be designed to meet unique weight, weatherproofing, and durability requirements, unique to each study species. To the best of our knowledge, widely available, affordable, and reliable proximity loggers for small mammals do not yet exist (see Boyland et al., 2013; Drewe et al., 2012 for discussions of the reliability of commercially available proximity loggers for larger animals).

Here, we tested a prototype of a miniaturized proximity logger called ProxLogs (Kirkpatrick et al., 2021) on a small, socially monogamous, semi-fossorial mammal: the prairie vole (*Microtus ochrogaster*). Prairie voles are a highly developed model for the behavior and evolution of social behavior, pair bonding, and monogamy (Carter & Getz, 1993; Insel et al., 1995; Shapiro & Dewsbury, 1990). They are a suitable model species for the testing of ProxLogs because researchers have studied them for decades through trapping, passive monitoring, and VHF radio telemetry—therefore, we are privy to ample knowledge of both their social behavior and commonly used methods for the attachment of animal-borne devices. The sociability of prairie voles, including group-living, offers an opportunity to test the durability of the ProxLogs devices and their packaging, because voles may remove or chew on the attached ProxLogs of their social partners. Additionally, prairie voles spend much of their time in grass runways and underground burrows, which necessitates streamlined attachment and exceptional durability of the ProxLogs packaging. As proximity loggers are designed to explicitly measure social behavior and interactions, it is imperative to ensure that the equipment is suitable for this purpose.

In this study, we navigated the aforementioned challenges to ProxLogs deployment in prairie voles and compared several generations of packaging models. We ultimately developed and refined a suitable packaging option for proximity loggers on small, social mammals that have historically been difficult to directly observe. We compared the resolution and consistency

of our recorded ProxLogs data to 1) social contact data collected through traditional live trapping and 2) a passive recording method for collecting social behavior in prairie voles. We demonstrate proof of concept for the use of proximity loggers on small, terrestrial mammals, discuss the efficacy of each method, and provide recommendations for the further use of proximity loggers in recording fine-scale social contact data in small mammals.

2.3 Methods

2.3.1 Study Area

We conducted all fieldwork for the study at the Miami University Ecology Research Center (ERC) in Oxford, Ohio (39° 53'N, 84° 73'W), over 21 months (Sep 2020–Jun 2022), using one existing semi-natural, enclosed, outdoor population established in June 2019 (for details of founding population, see **Chapter 3** of this dissertation). The enclosure was 0.1 ha (32 m x 32 m), surrounded by 20-gauge galvanized steel walls that extended 75 cm high and 45 cm below-ground to prevent voles from escaping (Cochran & Solomon, 2000). To exclude terrestrial predators from the study area, an electric wire ran along the top of the walls of the enclosure. However, because the enclosure was uncovered on top, voles could experience bird or snake predation, as they do in their natural habitat. We regularly mowed a 1-m wide strip at the inner edge of each enclosure wall to discourage digging and routinely checked that the walls were in good condition.

The vegetation in the enclosure consisted of goldenrod (*Solidago* spp.), clover (*Trifolium* spp.), timothy (*Phleum* spp.), ryegrass (*Elymus* spp.), fescue (*Festuca* spp.), bluegrass (*Poa pratensis*), and ragweed (*Ambrosia* spp.), which provided natural food and cover for voles (Solomon et al., 2009; **Supplementary Table 2.1**). Woody and thorny plants such as brambles (*Rubus* spp.) and common teasel (*Dipsacus fullonum*) were observed and removed over the

course of the study, to maintain resemblance of the enclosures to the native grassland prairie habitat of prairie voles. These plants made up less than 15% of vegetation (**Supplementary Table 2.1**). We occasionally caught bycatch such as deer mice (*Peromyscus* spp.; N = 16) or short-tailed shrews (*Blarina brevicauda*; *N* = 4), which were immediately released outside of the enclosure without handling.

2.3.2 Study Animals

In September 2020, when we began collecting data on the individuals included in this study, the total population density was 13 voles (8 females; 11 males; 1 unrecorded), equivalent to a medium-high population density of 130 voles/ha, according to previous long-term studies of the natural fluctuations in vole densities in the wild (Getz et al., 1993, 2001). Founding animals of these existing populations were introduced in 2019, as 76 adult laboratory-bred prairie voles (*Microtus ochrogaster*), descended from voles originally captured in Illinois and bred by the Angela Grippo Laboratory at Northern Illinois University and the Alex Ophir Laboratory at Cornell University (full details and methods provided in **Chapter 3** of this dissertation). All voles in the study were marked with passive integrated transponder (PIT) tags (Biomark: Boise, Idaho; 12mm HPT tags) when they reached a juvenile stage at a weight heavy enough to safely tag (12 g; i.e., 12 days old; MD personal observation). Animals were not food-supplemented while in the enclosures and received only the cracked corn used to bait traps, which is a low-nutrient food source that adds little caloric value to their diet (Desy & Batzli, 1989). The animals were allowed to breed naturally within the outdoor enclosure until the end of the study in June 2022, when all animals were removed from the enclosures via live trapping and humanely euthanized.

2.3.3 Live Trapping

We used live trapping (hereafter, "trapping") during this study to collect social interaction data and capture offspring for PIT tagging. We set up traps on a 5 m x 5 m grid in each enclosure, with three multiple-capture Ugglan traps (Granhab: Gnosjö, Sweden) at each grid location (**Figure 2.1**). Therefore, during each trapping session, approximately 75 traps were set in each enclosure. We recorded the PIT tag identities of every animal captured in each trap.

We trapped in each enclosure at least once per week (Sun–Sat) during the peak breeding seasons to ensure continuous data collection and to catch as many offspring as possible, while seeking to minimize disturbance to the populations. During the peak breeding season, we conducted either evening sessions (setting traps at approximately 1700 h and checking them at approximately 2000 h) or overnight sessions (setting traps at approximately 2000 h and checking them at approximately 0600 h). This trapping procedure minimized the amount of time animals spent in traps, while taking advantage of the time of day when prairie voles are most active (1800–2000 h; Sabol et al., 2018). We did not trap during heavy rain or thunderstorms and rescheduled missed trapping sessions when possible.

During late autumn–early spring (approximately Oct–Apr), we trapped at least once every 30 days to monitor populations during these colder months. Monthly trapping has previously been validated as an effective time interval for monitoring population density, survival, and sex ratio (Getz et al., 2006). In a previous study, calculations of these three variables through monthly trapping sessions closely tracked the patterns shown by 3.5-day trapping intervals, demonstrating that more frequent trapping does not significantly increase the amount of demographic information recorded, but does increase disturbance of the animals (Getz et al., 2006). When the ambient temperature dropped below 5 °C overnight, we trapped during

the day (approximately 0800–1800 h) and checked traps every 1–2 hours, to avoid and mitigate animal exposure to cold temperatures.

2.3.4 Radio Frequency Identification (RFID) Data

We dispersed 12 RFID antennas throughout the enclosure in a 3 x 4 array, with antennas at a distance of 8 m horizontally (across the rows of antennas) and 7.33 m vertically (across the columns of antennas; see **Figure 2.1** for details). These antennas were connected to a central Reader (Biomark: Boise, Idaho; RM310/SM303 System), which detected and recorded the PIT tags of any vole within 1–3 cm of the antenna surface (MD, personal observation) once per second (Sabol et al., 2018). As animals moved throughout the enclosure, the RFID antennas recorded a random sampling of their location at certain times, which we used to estimate behavior. The RFID antennas we used can record only one PIT tag at any given time, due to signal collision (MD, personal observation; Biomark: Boise, Idaho, personal communications). Therefore, we used the lag time between the consecutive detection of two tags at the same antenna to develop our social networks from spatial and temporal overlap (methods detailed in *2.3.7 Construction of Social Networks*).

2.3.5 ProxLogs Collar Design

In this study, we aimed to test a method for recording direct social interactions, which should provide higher-resolution affiliation data than either trapping or RFID. We used a proximity logger system (ProxLogs) being developed by Kirkpatrick et al. (2021) that uses Bluetooth low-energy (BLE) technology. In this system, individuals are tagged or collared with animal-borne ProxLogs devices, which detect a contact when two loggers are within 5–6 cm of each other (MD, unpublished data). The advertising and scanning rates of the loggers can be

adjusted to increase either data resolution or battery life, as a trade-off. These loggers have been

designed with an emphasis on user flexibility (Kirkpatrick et al., 2021), so the attachment

method must be designed by the researcher using the ProxLogs system to suit their study species.

Collaring prairie voles with VHF radio-telemetry units has been common in field

mammalogy research, from the 1980s (e.g., Getz & Hofmann, 1986) to today (e.g., Rice et al.,

2022). These devices generally weigh 1.9–3.0 g (e.g., Rice et al., 2022; Lambert et al., 2021;

respectively), which has not previously affected energy use in voles (Berteaux et al., 1996; but

see Casper, 2008 and Portugal et al., 2018 for more recent discussions of biologger effects on

energy use). Using the conventional radio collar design (e.g., Holohil: Carp, Ontario, Canada) for

prairie voles as a model, we aimed to engineer zip tie collar packaging that was easily

rechargeable, relatively lightweight, low-profile, waterproof, and resistant to chewing by

conspecifics. We tested six designs, which were iterations of three concepts: 1) custom 3D

printed casing, 2) dual wall heat shrink casing, and 3) UV-curing epoxy resin casing. Notably,

we only deployed collars designed with dual wall heat shrink casing and UV-curing epoxy resin

casing, because the 3D printed casing was too bulky to deploy (see *2.3.5.1 Custom 3D-Printed*

Casing). Additionally, because we wanted to reduce weight and surface area, we packaged

versions of the collars with the manufactured charging pins intact and with the charging pins

replaced with aftermarket metal rings (**Figure 2.2**). To replace the pins, we unsoldered the pins

and soldered the rings in place on the same charging pads.

2.3.5.1 Custom 3D-Printed Casing

The first collar design we tried was a custom 3D-printed casing made of Polylactic Acid

(PLA) filament, created using the exact specs of the logger in Autocad Fusion 360 (**Figure 2.3**

A). The ProxLogs device was placed into a compartment in the casing and silicon was filled into

the empty space to provide protection and waterproofing. The design included a lid which slid into place and could be sealed with silicone to ensure waterproofing (**Figure 2.3 A**). Finally, a slot on the lid provided a place for us to thread a zip tie through and secure the packaged ProxLogs device to the animal (**Figure 2.3 A**). To recharge the 3D-printed collars, we had to pry the lid off of the casing and gently remove the silicon from the casing compartment to free the logger. However, we abandoned this method due to its large size without deploying the loggers, so we did not have the opportunity to recharge and reuse them.

2.3.5.2 Dual Wall Heat Shrink Tubing Casing

The second generation design we tested used dual wall heat shrink tubing (hereafter, "heat shrink tubing") to cover and provide a waterproof seal for the ProxLogs unit, without the bulk of the 3D-printed case (**Figure 2.3 B**). We used heat shrink tubing because it was more durable than elastidip (e.g., Kirkpatrick et al., 2021) to chewing by voles and easier to use than epoxy (Kirkpatrick, personal communication). It has also been used in other studies to wrap biologgers such as global positioning system (GPS) units and accelerometers (e.g., Cook et al., 2016; Houstin et al., 2022; Zhang et al., 2020).

We wrapped the ProxLogs unit in commercially available plastic wrap and cut a piece of 1.27 cm diameter adhesive-lined heat shrink tubing just longer than the logger (~ 0.32 cm on each side). We then inserted the ProxLogs unit into the length of tubing and used a lighter to shrink the tubing, melting the adhesive on the inside of the tubing. We clamped the edges shut while the adhesive dried, creating a seal from the glue on both ends. The first iteration of this collar used a flat piece of adhesive-lined shrink wrap to traverse the collar lengthwise and hold the zip tie to the collar (**Figure 2.3 B**). Our second iteration used super glue and two additional

lengthwise zip ties to hold the packaged ProxLogs unit to the zip tie, reinforced by electrical tape wrapped around the outside (**Figure 2.3 B**).

Our third iteration of this design had an additional inner layer of Kevlar (aramid) to protect the ProxLogs device from animals who might chew through the heat shrink tubing. In short, we wrapped the ProxLogs device in plastic wrap, aramid, and then applied the heat shrink tubing on the outside to create the waterproof seal. We used super glue and electrical tape to secure the packaged ProxLogs device to the zip tie collar. To recharge the heat shrink tubing collars, we used scissors to carefully break the seal on one end of the packaging and peel the heat shrink tubing from the outside of the Proxlogs device. When the device was recharged, we repeated the entire packaging process.

2.3.5.3 UV-Curing Epoxy Resin Casing

Finally, we used a UV-curing epoxy resin (hereafter, "epoxy resin") to create a more durable collar packaging (**Figure 2.3 C**). This type of resin is liquid until it comes into contact with UV light, at which point it hardens into an epoxy. Here, we created a rectangular mold from polymer clay set onto a clear piece of plastic. We poured a thin layer of the epoxy resin into the mold and set it with a UV light. We then wrapped the logger in plastic wrap, set it into the mold (with the charging pins stuck into the clay, if applicable), and used the epoxy resin to fill the rest of the mold. When the epoxy resin around the logger hardened under the UV light, it created a durable casing around the outside of the logger. At this point, we removed the clay and used the UV light to set each of the sides that had not yet been exposed.

After the creation of this rectangular casing, we used a Dremel (rotary tool) to thin the epoxy resin and shape the collar into a round, streamlined shape. At this point in the process, we could remove and add additional resin in stages to adapt each collar's size and shape as needed.

We added a visible number label to each collar to keep track of them without using the phone application (hereafter, "app"), which was designed (for iPhone or Android operating systems) to turn ProxLogs on and off, adjust ProxLogs settings, and download data from ProxLogs as .csv files, to check the number. At this point, if applicable, we covered the exposed charging pins with a small piece of medical intravenous (IV) tubing, stuffed any empty space with plastic wrap, and covered it all with a thin layer of epoxy resin. Finally, we attached the zip tie using epoxy resin.

Our second iteration of this design added sand to reinforce the vulnerable edges of the logger around the charging pins. We used this method to deter chewing on the collars by voles, because research has shown that textural repellants such as sand mixed into paint can deter beavers from chewing on established trees (Nolte et al., 2003). In short, we put a thin layer of epoxy resin along the bottom of the logger, dipped that edge in sand, and cured the resin. We then knocked off any excess sand and put a light second layer of resin to create a smooth surface on the collar. During this process, we shaved the layer of resin underneath the sand closer to the device so that the sand did not add weight to the final packaged logger.

To expose the charging pins and recharge the epoxy resin collars, we used wire-cutters or pliers to crack the thin layer of epoxy on the pins and slip off the medical IV tubing. Then, when charged, we added a new piece of medical IV tubing to the outside of the pins and covered it with resin (and sand, when applicable).

2.3.6 ProxLogs Deployment and Data Collection

ProxLogs were deployed on animals in December 2021 (n = 22), March 2022 (n = 5), and May 2022 (n = 6) (see **Supplementary Table 2.2** for full details of deployment). Prior to deploying the loggers, we used the phone app to turn them on and set their scanning and

advertising rates. To deploy them, we looped the end of the zip tie and pulled it over the vole's head, using one person to hold the animal and the other person to position the collar. We then tightened the collar around the animal's neck until it was tight enough to not slip over the head and loose enough that the animal could comfortably breathe and move around. Animals were observed for approximately 15 minutes prior to release, to verify that they were able to move around freely without any restrictions.

Each time thereafter that we caught a collared animal, we would download the data as a .csv file from the collar using the ProxLogs phone app. If the collar did not show up on the app, we would remove it, check the animal's neck to ensure there was no chafing or discomfort, and either release the animal immediately or replace the collar with a new one. We charged the dead collars and repackaged them before deploying them on another animal. In some cases, the collar was damaged beyond repair and could not be reused. The .csv file downloaded from each collar contained the date and time of contact, the collar ID, the ID of the other collar recorded during the contact, and a "distance" value indicating the proximity of the two collars when the contact was recorded, as a Received Signal Strength Indicator (RSSI). An example of a downloaded .csv file is shown in **Figure 2.4**. A collar could carry up to 4000 logs in memory before it stopped logging; therefore, 4000 was the maximum number of social contacts reported in a downloaded .csv file. Loggers were usually filled within 24–48 hours, although the battery life of the loggers was approximately 5–6 days with 30 s advertising windows (MD, personal observation).

2.3.7 Construction of Social Networks

For our analyses, we created three networks consisting of the same individuals as nodes: one network using the trapping data, one network using the RFID data, and one network using the ProxLogs data. All analyses were performed in R version 3.6.1; social networks were

constructed using the R packages asnipe (version 1.1.16; Farine, 2017) and igraph (version 1.5.1;

Csardi & Nepusz, 2006). We considered events when animals were present at the same trapping

location (i.e., trapping stake; details in *2.3.3 Live Trapping*) or in the same trap together during a

trapping session (i.e., spatial-temporal co-occurrence) to be social contacts (i.e., social network

edges; Solomon et al., 2009). Similarly, we used each detection event generated by the ProxLogs

as social contact events because the animals must have been within 5–6 cm of each other for the

contact to occur (MD, unpublished data).

We ran the raw temporal stream of individual RFID detections through a Gaussian

mixture model (GMM), which used antenna location and time in seconds from the start of the

recording period to create grouping events based on spatial and temporal overlap (Psorakis et al.,

2012). The output data were organized into a matrix of K groups x N individuals, such that

individuals placed by the model into the same group (range: 6–81551 seconds; median: 2686

seconds; mean: 8989 seconds; **Figure 2.5**) had pairwise social contacts. These contacts were

weighted by their simple ratio index (SRI), which is the proportion of the number of times that a

pair was recorded *together* to the *total* number of times each individual was recorded (Cairns &

Schwager, 1987; Farine, 2013). For example, if two individuals were detected 10 seconds apart

by the same RFID antenna, these individuals would likely be grouped together by the GMM,

based on spatial-temporal co-occurrence. These voles would then be assigned a social network

edge, weighted by the number of times that same pair of individuals was observed together in

separate instances, the number of times each individual had been detected by itself or with

another partner of either sex, and the number of times that both individuals had been observed by

different antennas at the same time (i.e., temporal but *not* spatial co-occurrence; Hoppitt and

Farine, 2018). There is high variability in the range of lag times between detections considered to

be social contacts by our GMM, but we did not threshold the data (i.e., assign a cutoff for assigned contacts to be considered valid, such as within 10 min). The benefit of using a GMM for detecting likelihood social contacts is that researchers do not have to set an arbitrary time threshold or cutoff—the algorithm determines this cutoff through permutations of the raw data stream (Psorakis et al., 2012, 2015). See **Figure 2.5** for the full distribution of these data.

In each network, the number of unique edges that each individual has is known as the unweighted degree, and each edge is weighted by adding the weights of the time-ordered edges between two individuals within the aggregated time period. Simply put, the unweighted degree represents the *number of unique individuals* that the focal individual is associated with, and the degree weight represents the *strength* of that association.

2.3.8 *ProxLogs Packaging*

We compared the efficacy of packaging methods by measuring the proportion of deployed ProxLogs that were 1) recovered and 2) recovered intact, without being damaged. We used chi-square tests to determine whether these proportions were statistically different between collars packaged with heat shrink tubing and collars packaged with epoxy resin.

2.3.9 *Method Comparison*

To address the suitability of ProxLogs for use on small mammals, we first compared the total number of individual detections. In other words, we determined how many times each individual was recorded by trapping, RFID, and ProxLogs to understand the resolution of demographic and movement data (either alone, or with another individual). We also compared the number of "paired" detections as spatial-temporal co-occurrence events (i.e., animals trapped

at the same location or trap during the same trapping session, RFID contact events determined by the GMM, and ProxLogs detections).

To do this, we ran a Friedman test using the R package rstatix (version 0.7.2; Kassambara, 2023), which allowed us to test whether there were differences in the mean number of individual or paired detections. We used the Friedman test because it is a nonparametric alternative to a repeated measures ANOVA, and our data did not meet the normality assumptions of a repeated measures ANOVA (tested using a Shapiro-Wilk test and Q-Q plot). We then ran a post-hoc Wilcoxon signed rank test with a Bonferroni correction to measure the paired differences between the means of our groups.

We had few (N = 8) paired recordings using RFID over the time period of this study, so we combined these measurements with paired recordings from live trapping and compared this combined data set to the ProxLogs data set. For this study, we were interested in measuring fine-scale, dynamic social behavior, so it was crucial to know whether there were differences in the number of times that vole pairs were recorded, or whether the distribution of these recordings varied. For example, many recordings with high variability in the members of each paired detection would have different implications than a large number of recordings between the same two individuals. Here, we used a sign test with a Bonferroni correction to compare ProxLogs detections and combined trapping and RFID detections, as a nonparametric alternative to a paired t-test, because again, our data did not meet the normality assumptions of a paired t-test (tested using a Shapiro-Wilk test and Q-Q plot).

Once we had performed these basic exploratory analyses regarding the temporal resolution of data recorded by each method, we conducted social network analyses (SNA) to determine whether there were consistent differences in the number of unique connections being

recorded between individuals (i.e., unweighted degree) or the strength of these connections (i.e., degree weight). Here, we compared traditional trapping methods to ProxLogs, because the GMM detected few social contacts between individuals using the RFID data (N = 8). See graph depictions of both social networks in **Figure 2.6**. We summarized the number of unique connections and connection strength from the social networks described in *2.3.7 Construction of Social Networks*, such that each individual was assigned an unweighted degree and degree weight for both trapping and RFID methods. In some cases, an individual was either not detected by one method or not detected with a social partner; they were assigned both an unweighted degree and degree weight of zero. We then used a sign test with a Bonferroni correction to compare the means of unweighted degree and degree weight, measured by trapping and ProxLogs.

Finally, we used a Multiple Regression Quadratic Assignment Procedure (MRQAP) with double semipartialling (DSP; Dekker et al., 2007) to determine whether the connections detected using our trapping network predicted the connections we recorded using ProxLogs. In short, MRQAP allows regression of matrices to determine the influence of one matrix on another, in the way that linear regression calculates the effects of predictor variables on the response variable. MRQAP DSP also uses permutation to estimate and calculate the effects of error (Dekker et al., 2007). For these analyses, we used the association matrix of the ProxLogs network as the response variable and the association matrix of the live trapping network as the predictor variable. Both matrices included connections that were weighted using the SRI.

2.3.10 Ethical Note

All research procedures and methods were approved by the Institutional Animal Care and Use Committees (IACUC) at University of Michigan (protocol #PRO00010760) and Miami

University (protocol #979) and performed in accordance with the American Society of

Mammalogists guidelines for the use of wild mammals in research (Sikes et al., 2016).

2.4 Results

2.4.1 ProxLogs Packaging

There were no significant differences between the proportion of ProxLogs packaged with

heat shrink tubing or epoxy resin retrieved after deployment (chi-square test, $X^2 = 3.29$; $p = 0.10$;

Figure 2.7 A). However, we retrieved 73% more intact ProxLogs when we packaged them with

epoxy resin than we did when we packaged them with heat shrink tubing (chi-square test, $X^2 =$

16.31; $p < 0.0001$; **Figure 2.7 B**). As such, we were able to download the data and reuse the

devices significantly more often when we used epoxy resin to package them. Adding sand to the

epoxy resin increased the percentage of intact collars retrieved to 100%, but this was not a

significant increase from the 82% success rate of the epoxy resin packaging without sand ($p =$

1.0).

2.4.2 Methods Comparison

2.4.2.1 Data Resolution

When comparing the number of individual detections recorded by traditional trapping

methods, passive recording by the RFID reader, and contacts between ProxLogs, we found that

the ProxLogs recorded, on average, 97% more total detections than RFID ($p < 0.001$; **Figure**

2.8) and 99.99% more total detections than trapping ($p < 0.001$; **Figure 2.8**), with ProxLogs

recording a median of 4,349 more social contacts than RFID or trapping (large effect size;

Kendall's W = 0.547).

ProxLogs also recorded 99.7% more paired detections (i.e., social contacts calculated by spatial-temporal co-occurrence) than the combination of trapping and RFID ($p = 0.01$; **Figure 2.9**). The median difference between the number of paired detections using ProxLogs and the combination of co-occurrence of trapping and RFID was 3,495 contacts.

2.4.2.2 Social Network Analyses

Average unweighted degree summarized from social networks constructed with proximity logger data was higher than average unweighted degree summarized from social networks constructed using trapping data ($p = 0.01$; **Figure 2.10**). Average degree weight, calculated using SRI, was also higher when constructed with proximity logger data as compared to trapping data (p = 0.004; **Figure 2.11**).

2.4.2.3 *Multiple Regression Quadratic Assignment Procedure*

The association matrix generated using trapping data significantly predicted the association matrix generated with proximity logger data (p = 0.007). There remained a lot of unexplained variation present in the data ($R^2 = 0.02$), suggesting remaining noise or systematic differences in the methods. However, because there were such huge differences in the amount of data being collected by each method, we would not expect that the low-resolution (trapping) data would be able to explain much of the variation in the high-resolution (ProxLogs) data.

2.5 Discussion

Over the course of this study, we were able to use epoxy resin to develop a proximity logger packaging that was both suitable to our needs and durable for long term use. While the use of sand to deter chewing on the edges of our packaged ProxLogs did not significantly improve retrieval rates of the intact devices for data download and reuse, it eliminated physical

damage to the cases of the ProxLogs (**Figure 2.7**). Therefore, to maximize data collection and budget efficiency, we recommend adding a textural repellant (like sand) to the packaging material when using animal-borne proximity loggers on study species that have high rates of social grooming. Because prairie voles are highly social and semi-fossorial, our collars needed to be resistant to chewing by a social partner and abrasions from soil; however, durability could be reduced to save logger weight in study species that are less social and spend their time aboveground (e.g., the use of elastidip; Kirkpatrick et al., 2021). Our development of a proximity logger packaging method for a mammal with these challenging requirements suggests that these devices could be widely used across other species of small mammals—an advancement in field methods that could improve our overall understanding of social behavior and its dynamics.

　　We found that on all measures, ProxLogs collected data at higher resolutions than RFID or traditional trapping methods (**Figure 2.8**; **Figure 2.9**; **Figure 2.10**; **Figure 2.11**). Importantly, with the ProxLogs system that we deployed, all detections of individuals were also paired detections because collar IDs were only recorded when two or more voles encountered each other. However, systems such as ProxLogs have the capability to set up stationary loggers that can be placed throughout the study area. These stations would play the same role as RFID antennas, by detecting individuals near to or passing by the station. This type of deployment would add a spatial aspect to the data and allow for individual detection of collared animals in the absence of a social partner. In essence, a system that combined animal-borne proximity loggers and stationary proximity loggers would simultaneously collect the spatial data that the RFID system records *and* record direct social interactions. Due to technical difficulties with the prototype of the ProxLogs system that we used, we were unable to use the stationary loggers that we ordered, but this possibility offers a promising avenue for future research.

We found that the strength of the social contacts measured with ProxLogs was higher than the strength of the social contacts measured with trapping, to an amplified and more significant degree than the differences in the number of social contacts measured with these two methods (**Figure 2.10**; **Figure 2.11**). This result suggests that ProxLogs were measuring *repeated detections with the same social partners*, rather than an overall higher number of close contacts with all members of a population. This finding indicates that the use of proximity loggers could add certainty to the determination of monogamous pairs or social partners, as we know from previous research that bonded pairs of prairie voles spend much of their time in huddling or side-by-side contact (Shapiro & Dewsbury, 1990). Although we did not measure partner preference in this study for biological validation, these results mirror those of biologically validated proximity logger studies in larger animals. For example, proximity loggers have been used to quantify maternal behavior of cows (*Bos taurus*) to their calves and identify cow-calf pairs within a herd (Kour et al., 2021; Swain & Hurley, 2007), determine mating pairs of island foxes (*Urocyon littoralis*; Ralls et al., 2013), and measure the mating patterns of brushtail possums (*Trichosurus vulpecula*; Weihong et al., 2005). To validate our results, future studies should record behaviors of ProxLogs-collared prairie voles in a semi-natural, indoor environment that allows direct visual observation of the collared animals (e.g., the experimental pen described in McGuire et al., 2014).

Historically, trapping methods have been the only way to detect social interactions in the field with a high degree of certainty. In other words, if two animals are observed together in the same trap, they *must*, by definition, be engaging in a direct interaction. Alternatively, the use of RFID antennas to passively record social contacts allows researchers to study social patterns with a higher spatial-temporal resolution, while reducing the impact of invasive high-frequency

trapping on the animals and the level of intensive human effort required (Sabol et al., 2018).

However, while the use of an RFID system provides an easily deployed method to passively

record social contacts, it only allows researchers to *infer* the likelihood of a social interaction

based on spatial and temporal overlap in a shared environment. As such, our early research

demonstrated that RFID methods are too sparse to provide the temporal resolution required for

analyzing fine-scale social behavior over time (MD, unpublished data).

Conversely, proximity loggers, such as the ProxLogs used here, provide a high level of

temporal resolution, allowing the determination of consistent and reliable social contacts.

Proximity loggers only detect a contact when two or more animals are at close ranges of 5–6 cm

(MD, personal observation)—a distance at which they must be directly interacting. Therefore,

proximity loggers provide a promising new method for use with small mammals to increase data

resolution and explore questions that require fine scale knowledge of social behavior, which have

been previously impossible to answer in naturalistic conditions.

To our knowledge, this is the first study to deploy and test proximity loggers on small,

semi-fossorial mammals in natural conditions. To modify these loggers for use in prairie voles,

our goal was to develop a packaging method that was 5–10% of vole body weight, low-profile to

enable voles to navigate comfortably throughout the grassland environment and underground

tunnels, and resistant to both inclement weather and chewing. After much testing, we determined

that ProxLogs encased in epoxy resin achieved these aims.

Here, we provided proof of concept that proximity loggers can be used to reliably

measure social behavior of small mammals. Proximity loggers increase the resolution and

efficiency of data collection on small mammal social behavior. They can collect much more data

than high-frequency trapping, while decreasing the amount of time that study animals spend in

traps and allowing researchers to record their behavior as they interact freely throughout their natural environment. However, as this technology develops further, changes can be made to improve the packaging and deployment methods. ProxLogs are designed to be amenable to many different study systems, so that researchers can adapt and create their own packaging and attachment methods. While our method works reliably in prairie voles, researchers should consider the aims and limitations of their own study systems when employing proximity loggers in small mammal research. One generalizable lesson, however, is that researchers interested in using proximity loggers on small study species should consider using smaller battery sizes, when possible, as the battery makes up much of the weight of a packaged logger.

2.6 Acknowledgements

2.7 References

Alberts, S. C., & Altmann, J. (2012). The Amboseli Baboon Research Project: 40 years of continuity and change. *Long-term field studies of primates*, 261-287.

Berteaux, D., Masseboeuf, F., Bonzom, J. M., Bergeron, J. M., Thomas, D. W., & Lapierre, H. (1996). Effect of carrying a radiocollar on expenditure of energy by meadow voles. *Journal of Mammalogy, 77*(2), 359-363. https://doi.org/10.2307/1382808.

Bonter, D. N., & Bridge, E. S. (2011). Applications of radio frequency identification (RFID) in ornithological research: a review. *Journal of Field Ornithology, 82*(1), 1-10. https://doi.org/10.1111/j.1557-9263.2010.00302.x.

Bosson, C. O., Islam, Z., & Boonstra, R. (2012). The impact of live trapping and trap model on the stress profiles of North American red squirrels. *Journal of Zoology*, *288*(3), 159-169. https://doi.org/10.1111/j.1469-7998.2012.00941.x.

Boyland, N. K., James, R., Mlynski, D. T., Madden, J. R., & Croft, D. P. (2013). Spatial proximity loggers for recording animal social networks: consequences of inter-logger variation in performance. *Behavioral Ecology and Sociobiology*, *67*, 1877-1890. https://doi.org/10.1007/s00265-013-1622-6.

Casper, R. M. (2009). Guidelines for the instrumentation of wild birds and mammals. *Animal Behavior, 78*(6),1477-1483. http://dx.doi.org/10.1016%2Fj.anbehav.2009.09.023.

Cochran, G. R., & Solomon, N. G. (2000). Effects of food supplementation on the social organization of prairie voles (Microtus ochrogaster). *Journal of Mammalogy, 81*(3), 746-757. https://doi.org/10.1644/1545-1542(2000)081%3C0746:EOFSOT%3E2.3.CO;2.

Cook, T. R., Gubiani, R., Ryan, P. G., & Muzaffar, S. B. (2017). Group foraging in Socotra cormorants: A biologging approach to the study of a complex behavior. *Ecology and Evolution, 7*(7), 2025-2038. https://doi.org/10.1002/ece3.2750.

Cords, M. (2012). The 30-year blues: What we know and don't know about life history, group size, and group fission of blue monkeys in the Kakamega Forest, Kenya. *Long-term field studies of primates*, 289-311.

Craighead, F, .Jr., & Craighead, J. (1966). Trailing Yellowstone's grizzlies by radio. *National Geographic* 252–267.

Dekker, D., Krackhardt, D., & Snijders, T. A. (2007). Sensitivity of MRQAP tests to collinearity and autocorrelation conditions. *Psychometrika*, *72*, 563-581. https://doi.org/10.1007/s11336-007-9016-1.

Delehanty, B., & Boonstra, R. (2009). Impact of live trapping on stress profiles of Richardson's ground squirrel (*Spermophilus richardsonii*). *General and Comparative Endocrinology*, *160*(2), 176-182. https://doi.org/10.1016/j.ygcen.2008.11.011.

Desy, E. A., & Batzli, G. O. (1989). Effects of food availability and predation on prairie vole demography: a field experiment. *Ecology, 70*(2), 411-421. https://doi.org/10.2307/1937546.

Drewe, J. A., Weber, N., Carter, S. P., Bearhop, S., Harrison, X. A., Dall, S. R., McDonald, R. A., & Delahay, R. J. (2012). Performance of proximity loggers in recording intra-and inter-species interactions: a laboratory and field-based validation study. *PLoS One*, *7*(6), e39068. https://doi.org/10.1371/journal.pone.0039068.

Durant, S. M., Bashir, S., Maddox, T., & Laurenson, M. K. (2007). Relating long-term studies to conservation practice: the case of the Serengeti Cheetah Project. *Conservation Biology*, *21*(3), 602-611. https://doi.org/10.1111/j.1523-1739.2007.00702.x.

Fetherman, E. R., Avila, B. W., & Winkelman, D. L. (2014). Raft and floating radio frequency identification (RFID) antenna systems for detecting and estimating abundance of PIT-tagged fish in rivers. *North American Journal of Fisheries Management*, *34*(6), 1065-1077. https://doi.org/10.1080/02755947.2014.943859.

Fletcher, Q. E., & Boonstra, R. (2006). Impact of live trapping on the stress response of the meadow vole (*Microtus pennsylvanicus*). *Journal of Zoology, 270*(3), 473-478. https://doi.org/10.1111/j.1469-7998.2006.00153.x.

Getz, L. L., Cole, F. R., & Verner, L. (1986). Effectiveness of multiple-capture live traps for field behavioral studies of microtine rodents. Bulletin of the Psychonomic Society, 24(1), 72-74. https://doi.org/10.3758/BF03330507.

Getz, L. L., & Hofmann, J. E. (1986). Social organization in free-living prairie voles, *Microtus ochrogaster. Behavioral Ecology and Sociobiology, 18*, 275-282. https://doi.org/10.1007/BF00300004.

Getz, L. L., Hofmann, J. E., Simms, L., & McGuire, B. (2006). Efficacy of monthly and semi-monthly trapping of small mammal populations. *The American Midland Naturalist, 155*(2), 422-427. https://doi.org/10.1674/0003-0031(2006)155[422:EOMAST]2.0.CO;2.

Harper, S. J., & Batzli, G. O. (1996). Monitoring use of runways by voles with passive integrated transponders. *Journal of Mammalogy*, *77*(2), 364-369. https://doi.org/10.2307/1382809.

Houstin, A., Zitterbart, D. P., Winterl, A., Richter, S., Planas-Bielsa, V., Chevallier, D., ... & Le Bohec, C. (2022). Biologging of emperor penguins—Attachment techniques and

associated deployment performance. *PLOS One, 17*(8), e0265849. https://doi.org/10.1371/journal.pone.0265849.

Ji, W., White, P. C., & Clout, M. N. (2005). Contact rates between possums revealed by proximity data loggers. *Journal of Applied Ecology*, *42*(3), 595-604. https://doi.org/10.1111/j.1365-2664.2005.01026.x.

Kassambara, A. (2023). rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.2, https://CRAN.R-project.org/package=rstatix.

Kirkpatrick, L., Hererra Olivares, I., Massawe, A., Sabuni, C., Leirs, H., Berkvens, R., & Weyn, M. (2021). ProxLogs: Miniaturised proximity loggers for monitoring association behaviour in small mammals. *BioRxiv*, 2021-02. https://doi.org/10.1101/2021.02.28.432842.

König, B., Lindholm, A. K., Lopes, P. C., Dobay, A., Steinert, S., & Buschmann, F. J. U. (2015). A system for automatic recording of social behavior in a free-living wild house mouse population. *Animal Biotelemetry*, *3*(1), 1-15. https://doi.org/10.1186/s40317-015-0069-0.

Kour, H., Patison, K. P., Corbet, N. J., & Swain, D. L. (2021). Recording cattle maternal behaviour using proximity loggers and tri-axial accelerometers. *Applied Animal Behaviour Science*, *240*, 105349. https://doi.org/10.1016/j.applanim.2021.105349.

Lambert, C. T., Lichter, J. B., Perry, A. N., Castillo, S. A., Keane, B., Cushing, B. S., & Solomon, N. G. (2021). Medial amygdala ERα expression influences monogamous behaviour of male prairie voles in the field. *Proceedings of the Royal Society B, 288*(1956), 20210318. https://doi.org/10.1098/rspb.2021.0318.

Levin, I. I., Zonana, D. M., Burt, J. M., & Safran, R. J. (2015). Performance of encounternet tags: field tests of miniaturized proximity loggers for use on small birds. *PloS one*, *10*(9), e0137242. https://doi.org/10.1371/journal.pone.0137242.

Lynn, S. E., & Porter, A. J. (2008). Trapping initiates stress response in breeding and non-breeding house sparrows Passer domesticus: Implications for using unmonitored traps in field studies. *Journal of Avian Biology*, *39*(1), 87-94. https://doi.org/10.1111/j.0908-8857.2008.04204.x.

Madrid, J. E., Parker, K. J., & Ophir, A. G. (2020). Variation, plasticity, and alternative mating tactics: revisiting what we know about the socially monogamous prairie vole. *Advances in the Study of Behavior*, *52*, 203-242. https://doi.org/10.1016/bs.asb.2020.02.001.

McGuire, B., Bemis, W. E., & Vermeylen, F. (2014). Parental behaviour of prairie voles (Microtus ochrogaster) and meadow voles (M. pennsylvanicus) in relation to sex of offspring. *Behaviour*, *151*(4), 535-553. https://doi.org/10.1644/06-MAMM-A-451R.1.

McGuire, B., Pizzuto, T., Bemis, W. E., & Getz, L. L. (2006). General ecology of a rural population of Norway rats (Rattus norvegicus) based on intensive live trapping. *The American Midland Naturalist*, *155*(1), 221-236. https://doi.org/10.1674/0003-0031(2006)155[0221:GEOARP]2.0.CO;2.

Meise, K., Krüger, O., Piedrahita, P., Mueller, A., & Trillmich, F. (2013). Proximity loggers on amphibious mammals: a new method to study social relations in their terrestrial habitat. *Aquatic Biology*, *18*(1), 81-89. https://doi.org/10.3354/ab00492.

Mennill, D. J., Doucet, S. M., Ward, K. A. A., Maynard, D. F., Otis, B., & Burt, J. M. (2012). A novel digital telemetry system for tracking wild animals: a field test for studying mate choice in a lekking tropical bird. *Methods in Ecology and Evolution*, *3*(4), 663-672. https://doi.org/10.1111/j.2041-210X.2012.00206.x.

Melchior, H. R., & Iwen, F. A. (1965). Trapping, restraining, and marking arctic ground squirrels for behavioral observations. *The Journal of Wildlife Management*, 671-678. https://doi.org/10.2307/3798540.

Morris, D. W. (2014). Can foraging behaviour reveal the eco-evolutionary dynamics of habitat selection?. *Evolutionary Ecology Research, 16*(1), 1-18.

Morris, D. W., & MacEachern, J. T. (2010). Sexual conflict over habitat selection: the game and a test with small mammals. *Evolutionary Ecology Research*, *12*(4), 507-522.

Nolte, D. L., Lutman, M. W., Bergman, D. L., Arjo, W. M., & Perry, K. R. (2003). Feasibility of non-lethal approaches to protect riparian plants from foraging beavers in North America. *USDA National Wildlife Research Center-Staff Publications*, 256.

Packer, C. (2019). The African lion: a long history of interdisciplinary research. *Frontiers in Ecology and Evolution*, *7*, 259. https://doi.org/10.3389/fevo.2019.00259.

Perry, S., Godoy, I., & Lammers, W. (2012). The Lomas Barbudal Monkey Project: two decades of research on Cebus capucinus. *Long-term field studies of primates*, 141-163.

Portugal, S. J., & White, C. R. (2018). Miniaturization of biologgers is not alleviating the 5% rule. *Methods in Ecology and Evolution, 9*(7), 1662-1666. https://doi.org/10.1111/2041-210X.13013.

Prange, S., Jordan, T., Hunter, C., & Gehrt, S. D. (2006). New radiocollars for the detection of proximity among individuals. *Wildlife Society Bulletin*, *34*(5), 1333-1344. https://doi.org/10.2193/0091-7648(2006)34[1333:NRFTDO]2.0.CO;2.

Ralls, K., Sanchez, J. N., Savage, J., Coonan, T. J., Hudgens, B. R., & Cypher, B. L. (2013). Social relationships and reproductive behavior of island foxes inferred from proximity logger data. *Journal of Mammalogy*, *94*(6), 1185-1196. https://doi.org/10.1644/13-MAMM-A-057.1.

Rehmeier, R. L., Kaufman, G. A., & Kaufman, D. W. (2006). An automatic activity-monitoring system for small mammals under natural conditions. Journal of Mammalogy, 87(3), 628-634. https://doi.org/10.1644/05-MAMM-A-220R2.1.

Rice, M. A., Wong, G. H., & Ophir, A. G. (2022). Impacts of spatial learning on male prairie vole mating tactics in seminatural field enclosures are context dependent. *Animal Behaviour, 191*, 57-73. https://doi.org/10.1016/j.anbehav.2022.06.011.

Ripperger, S., Josic, D., Hierold, M., Koelpin, A., Weigel, R., Hartmann, M., Page, R., & Mayer, F. (2016). Automated proximity sensing in small vertebrates: design of miniaturized sensor nodes and first field tests in bats. *Ecology and Evolution*, *6*(7), 2179-2189. https://doi.org/10.1002/ece3.2040.

Wallace, G., Elden, M., Boucher, R., & Phelps, S. (2022). An automated radiotelemetry system (ARTS) for monitoring small mammals. *Methods in Ecology and Evolution*, *13*(5), 976-986. https://doi.org/10.1111/2041-210X.13794.

Sabol, A. C., Close, W. L., Petrullo, L., Lambert, C. T., Keane, B., Solomon, N. G., ... & Dantzer, B. (2023). Sociality does not predict oral microbiome composition or diversity in free-living prairie voles. *Animal Behaviour*, *200*, 167-182. https://doi.org/10.1016/j.anbehav.2023.02.009.

Sabol, A. C., Lambert, C. T., Keane, B., Solomon, N. G., & Dantzer, B. (2020). How does individual variation in sociality influence fitness in prairie voles?. *Animal behaviour*, *163*, 39-49. https://doi.org/10.1016/j.anbehav.2020.02.009.

Sabol, A. C., Solomon, N. G., & Dantzer, B. (2018). How to study socially monogamous behavior in secretive animals? Using social network analyses and automated tracking systems to study the social behavior of prairie voles. *Frontiers in Ecology and Evolution, 6*, 178. https://doi.org/10.3389/fevo.2018.00178.

Sanderson, G. C. (1966). The study of mammal movements: a review. *The Journal of Wildlife Management*, 215-235. https://doi.org/10.2307/3797914.

Shapiro, L. E., & Dewsbury, D. A. (1990). Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of vole (*Microtus ochrogaster and M. montanus*). *Journal of Comparative Psychology, 104*(3), 268. https://doi.org/10.1037/0735-7036.104.3.268.

Sikes, R. S., & Animal Care and Use Committee of the American Society of Mammalogists. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy, 97*(3), 663-688. https://doi.org/10.1093/jmammal/gyw078.

Snijders, L., van Rooij, E. P., Burt, J. M., Hinde, C. A., Van Oers, K., & Naguib, M. (2014). Social networking in territorial great tits: slow explorers have the least central social network positions. *Animal Behaviour, 98*, 95-102. https://doi.org/10.1016/j.anbehav.2014.09.029.

Solomon, N. G., Richmond, A. R., Harding, P. A., Fries, A., Jacquemin, S., Schaefer, R. L., Lucia, K.E., & Keane, B. (2009). Polymorphism at the avpr1a locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Molecular Ecology, 18*(22), 4680-4695. https://doi.org/10.1111/j.1365-294X.2009.04361.x.

Swain, D. L., & Bishop-Hurley, G. J. (2007). Using contact logging devices to explore animal affiliations: quantifying cow–calf interactions. *Applied Animal Behaviour Science*, *102*(1-2), 1-11. https://doi.org/10.1016/j.applanim.2006.03.008.

Yang, L., Han, J., Qi, Y., Wang, C., Liu, Y., Cheng, Y., & Zhong, X. (2010, September). Revisting tag collision problem in RFID systems. In *2010 39th International Conference on Parallel Processing* (pp. 178-187). IEEE. https://doi.org/10.1109/ICPP.2010.27.

Zhang, V. Y., Williams, C. T., Palme, R., & Buck, C. L. (2020). Glucocorticoids and activity in free-living arctic ground squirrels: Interrelationships between weather, body condition, and reproduction. *Hormones and Behavior, 125*, 104818. https://doi.org/10.1016/j.yhbeh.2020.104818.

2.8 Tables and Figures

Figure 2.1: Diagram of the radio frequency identification array configuration and the trapping stakes where we set our traps (depicted as pink squares and black dots, respectively). We set three traps at each trapping stake. RFID arrays are shown larger than scale for image visibility–each RFID antenna is located at the center of a pink square on the diagram.
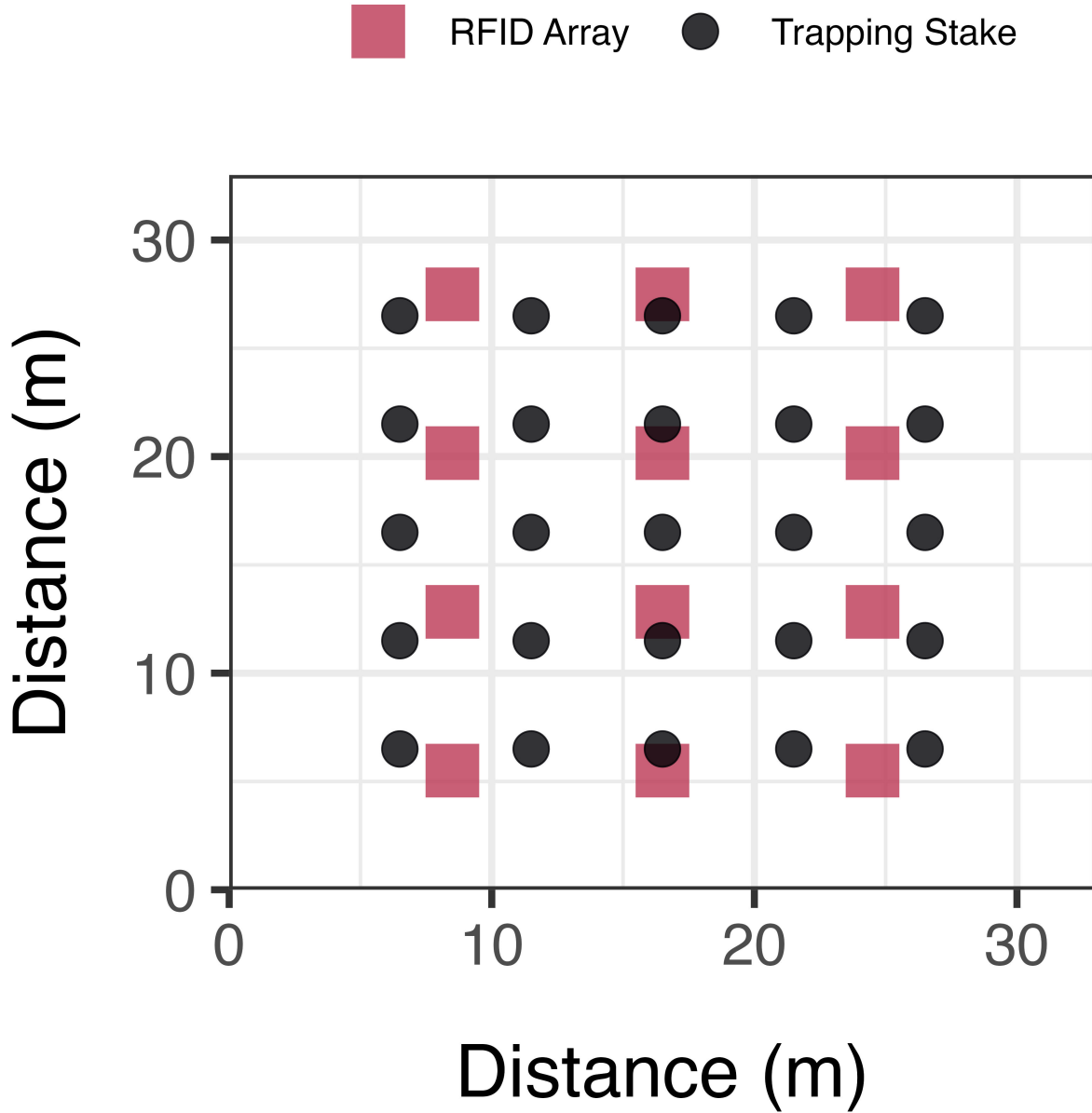
Figure 2.2: A side-by-side photo of two unpackaged ProxLogs: one with charging pins removed (left) and one with charging pins intact (right). We removed the pins to allow for more streamlined packaging, and to reduce the weight of the packaged ProxLogs.
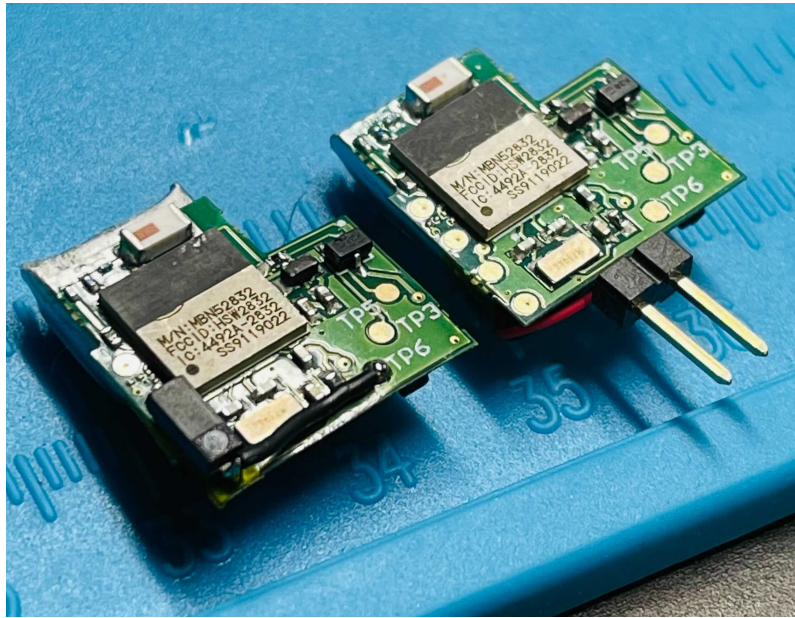
Figure 2.3: Photos of 3D-printed ProxLogs casing (A), heat shrink tubing ProxLogs casing (B), and UV-curing epoxy resin ProxLogs casing (C).

A 3D Printed Casing Design
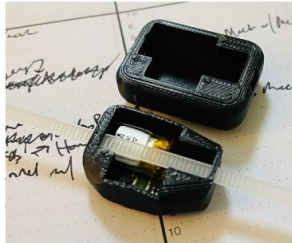
* Image provided for scale. Collar was removed immediately, prior to release of animal.

B Heat Shrink Tubing Designs

C UV Curing Epoxy Designs

55

Figure 2.4: An image of a .csv file downloaded from the ProxLogs phone application, with the collar number of the ProxLogs device that detected the contact ("id1"), the collar number of the ProxLogs device that was detected ("id2"), the date-time of the detection event ("datetime"), and the value indicating the proximity of the two collars when the contact was recorded, as a Received Signal Strength Indicator ("distance").

id1	id2	datetime	dist
22	53	19:58:55 15/12/2021	-78
22	36	20:47:55 15/12/2021	-66
22	36	20:48:05 15/12/2021	-72
22	36	20:48:35 15/12/2021	-74
22	36	20:48:45 15/12/2021	-73
22	36	20:48:55 15/12/2021	-73
22	36	21:19:15 15/12/2021	-62
22	37	21:55:55 15/12/2021	-78
22	37	21:58:25 15/12/2021	-74
22	37	22:50:24 15/12/2021	-68
22	37	22:50:35 15/12/2021	-72
22	37	22:50:45 15/12/2021	-63
22	37	22:51:04 15/12/2021	-73

Figure 2.5: Histogram of the time lag in seconds between individual RFID detections grouped into events by the GMM. The range of values is 6 - 81551 seconds, with a median of 2628 seconds (depicted as a vertical pink line) and a mean of 8908 seconds (depicted as a vertical purple line).
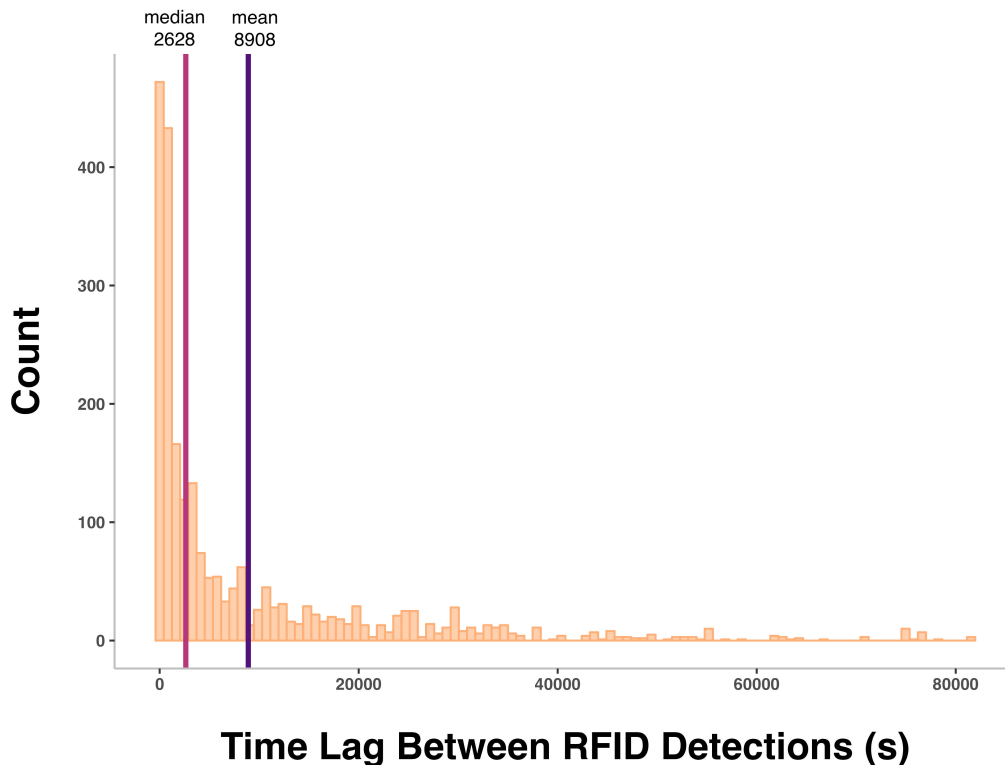
Figure 2.6: Social network graphs constructed with ProxLogs social contacts (A; nodes in light teal) and trapping social contacts (B; nodes in blue). Within each graph, circles are nodes, indicating each individual, and lines are edges, visualizing network connections. Edges (black lines connecting nodes) are weighted by the simple ratio index, so thicker lines represent social contacts that occurred more frequently, relative to the total number of observations of both individuals involved (i.e., Cairns & Schwager, 1987).
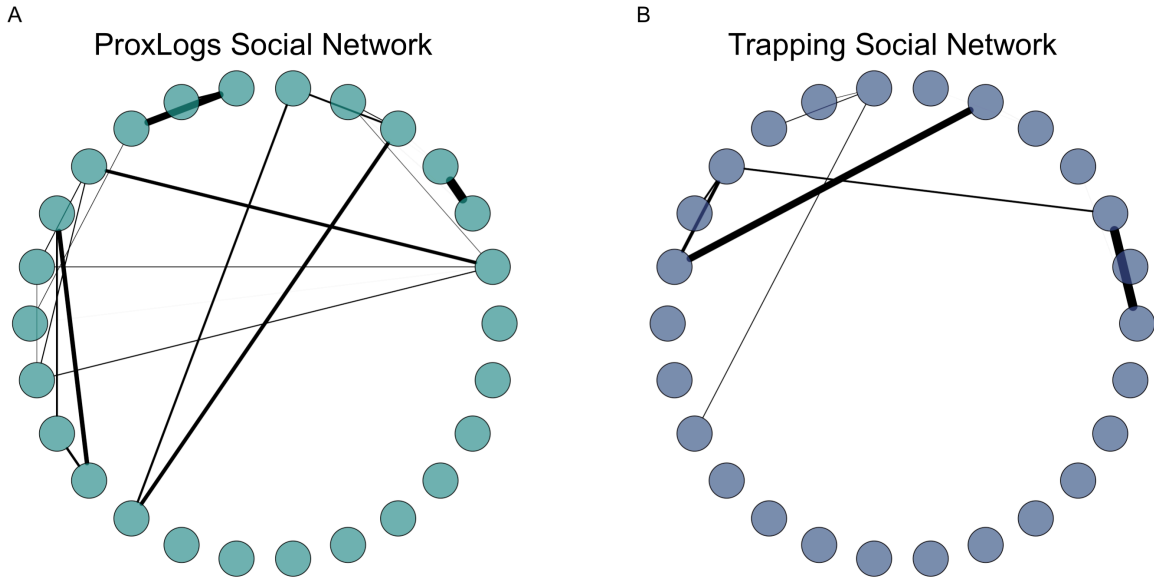
Figure 2.7: Proportion of deployed collars packaged with heat shrink tubing (light teal) and UV curing epoxy (blue) retrieved (A) and retrieved intact, without being broken (B). There was not a significant difference between the proportion of collars retrieved using each method ($p > 0.05$), but we retrieved significantly fewer intact collars using heat shrink, as opposed to UV curing epoxy ($p < 0.0001$). Zero loggers were retrieved in an intact state with the heat shrink tubing used as casing.
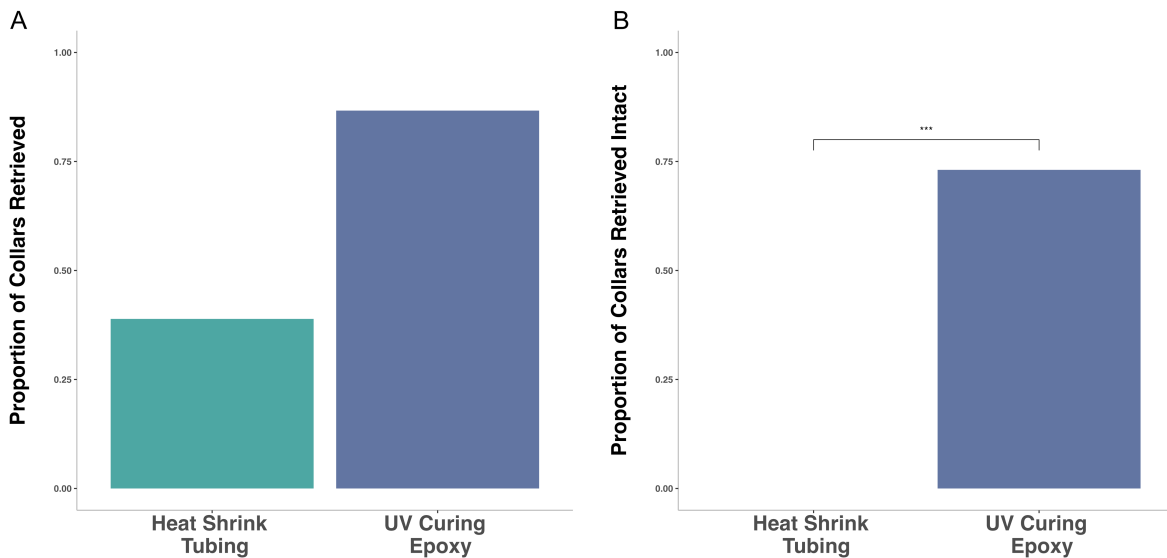
Figure 2.8: Total number of observations of individuals, recorded using ProxLogs (left, in yellow), RFID (middle, in teal), and trapping (right, in purple). ProxLogs recorded a significantly higher number of observations than both RFID ($p < 0.001$) and Trapping ($p < 0.001$). The y-axis is broken, with a gap from 1,500 observations to 17,300 observations, to more clearly show the distribution of observations from RFID and trapping.
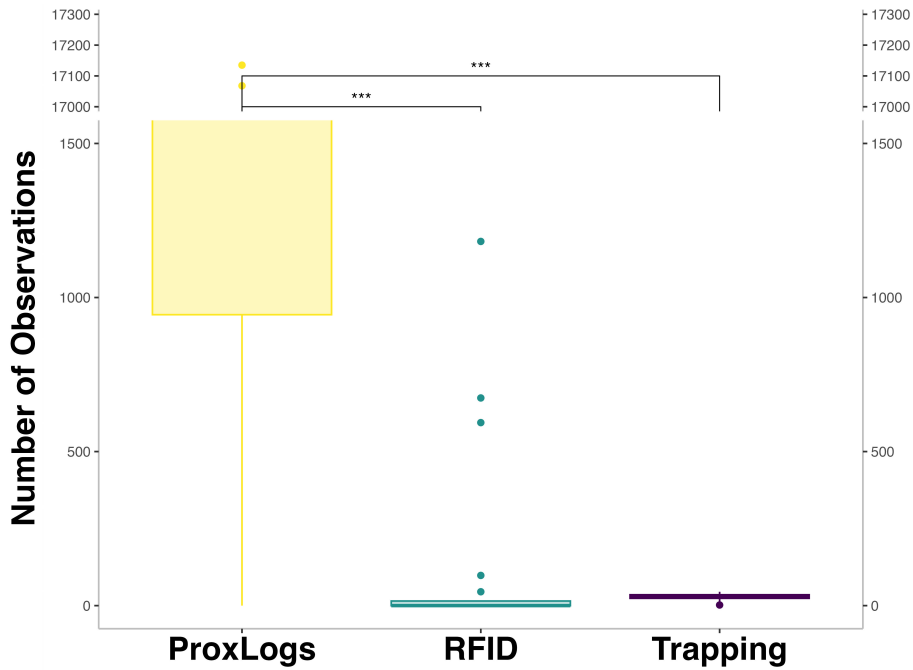
Figure 2.9: Total number of paired observations of two individuals by spatial-temporal overlap, recorded using ProxLogs (left, in yellow) or the combination of RFID and trapping (right, in purple). ProxLogs recorded a significantly higher number of observations than the combination of RFID and trapping ($p = 0.01$). The y-axis is broken, with a gap from 1,500 observations to 17,300 observations, to more clearly show the distribution of observations from RFID and trapping.
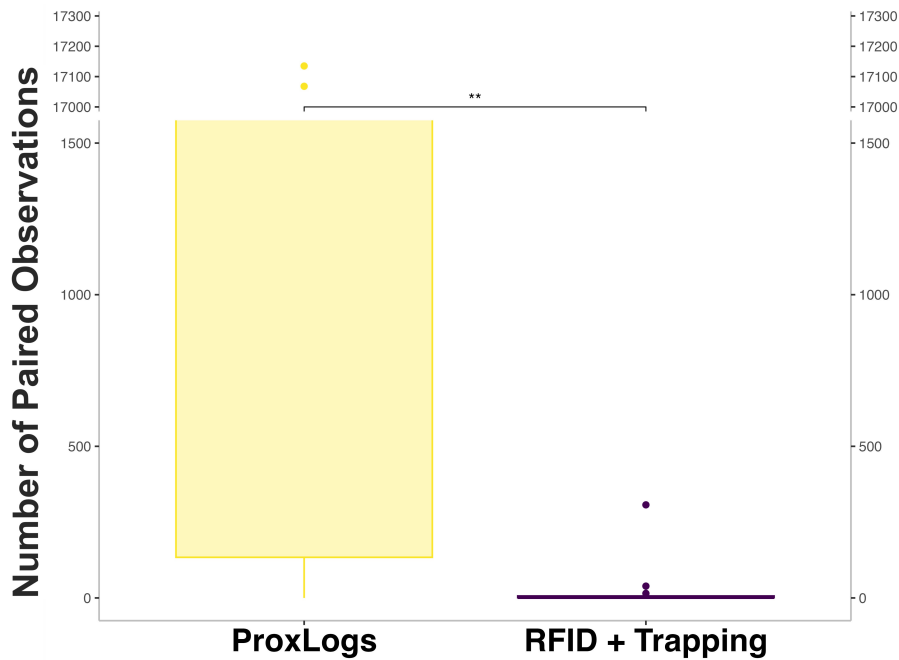
Figure 2.10: A boxplot showing the difference between unweighted degree summarized from a social network constructed with ProxLogs social contacts (left, in yellow) and a social network constructed with trapping social contacts (right, in blue). The horizontal lines between the points connect each individual's degree recorded with the two methods. ProxLogs recorded a significantly higher average unweighted degree than trapping ($p = 0.01$).
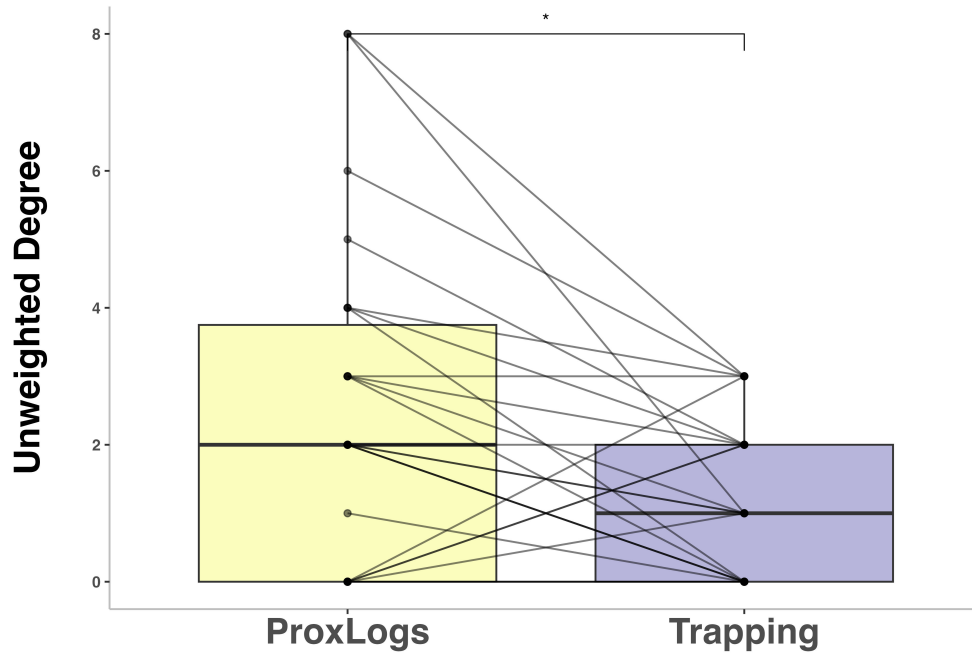
Figure 2.11: A boxplot showing the difference between degree weight summarized from a social network constructed with ProxLogs social contacts (in yellow) and a social network constructed with trapping social contacts (in blue). The horizontal lines between the points connect each individual's degree recorded with the two methods. ProxLogs recorded a significantly higher degree weight than trapping ($p = 0.004$).
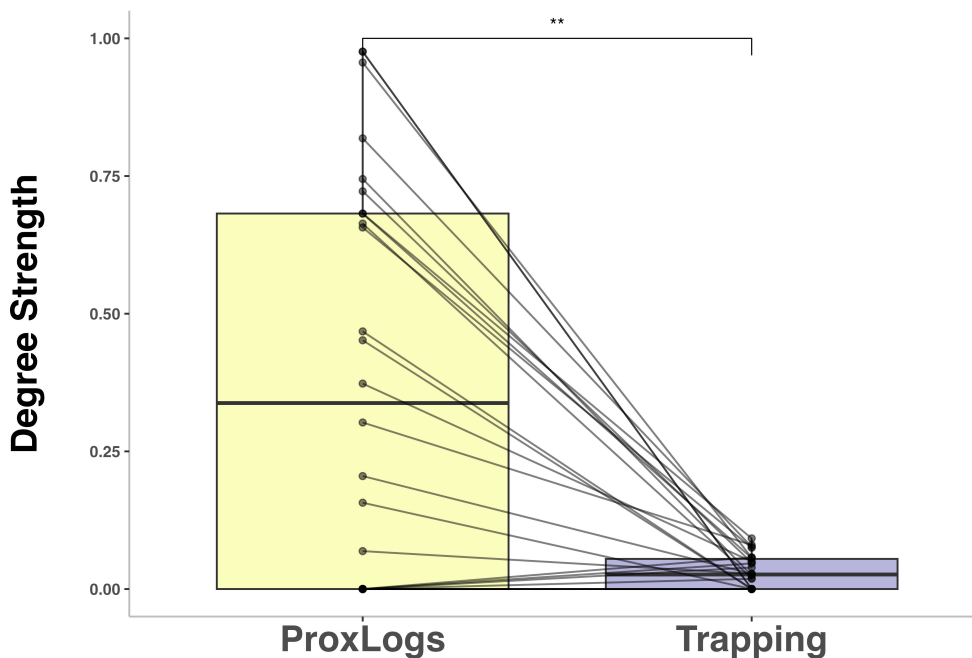
2.9 Supplementary Materials

Supplementary Table 2.1: Results of our 2021 vegetation survey, conducted from June 28–July 14, 2021, as part of a separate study. In short, random quadrats were selected to measure the percentage of surface area covered by vegetation, when viewed from above; the percentage of thorned plants (e.g., blackberry bushes [*Rubus fruticosus*], thistles [*Cirsium arvense*], common teasel [*Dipsacus fullonum*]); and the percentage of woody plants, following the protocol established and detailed in Lucia et al., 2008.

Enclosure	Quadrat	% Ground Covered	% Thorned	% Woody
1	31D	80	0	0
1	30C	75	0	15
1	16D	100	10	0
1	8A	100	0	0
1	2D	95	0	0
1	22A	100	0	35
1	9D	80	0	0
1	34A	100	0	0
1	24C	95	0	0
1	19B	100	0	0
2	22D	100	0	0
2	33C	100	15	0
2	28A	100	0	85
2	12D	100	0	0
2	9D	100	5	0
2	11D	100	15	0
2	25C	100	0	0
2	13B	100	10	0
2	3D	100	40	0
2	19B	100	5	0
3	33A	100	70	0
3	1D	90	0	0
3	32B	100	90	0
3	14B	100	0	0
3	21D	100	40	0
3	7A	100	0	0
3	34C	100	30	5
3	10B	100	0	0
3	30D	100	25	0
3	8C	100	0	0
MEAN		**96.88**	**11.77**	**3.02**

Supplementary Table 2.2: Summary of the number of ProxLogs collars deployed, retrieved, and retrieved intact from each collar type (A), and deployment details of ProxLogs, including numeric PIT tag ID for each vole collared, numeric collar ID for each collar deployed, and the dates that the collar was deployed and removed from each individual (B). Collars were considered intact when both the casing and internal logger were undamaged at retrieval.

A. Summary

Collar Type	Number Deployed	Number Retrieved	Number Intact
Heat shrink tubing	18	7	**0**
UV curing epoxy	30	26	**19**

B. Deployment

Tag	Collar ID	Date Collar Deployed	Date Collar Removed
C01E9EA	42	11/21/21	12/6/21
C01E9D1	41	11/21/21	6/5/22
C01EAE6	47	12/6/21	6/5/22
C01E98B	48	12/6/21	6/5/22
C01E9EA	49	12/6/21	6/5/22
C01E9CE	46	12/6/21	12/10/21
C004675	32	12/7/21	6/5/22
C01E966	33	12/7/21	6/5/22
C01EA00	34	12/7/21	12/16/21
C01E9EC	35	12/7/21	12/15/21
C01E95D	36	12/7/21	12/17/21
C01E982	37	12/7/21	12/17/21
C01EA00	53	12/12/21	12/17/21
C01E94D	43	12/15/21	12/17/21
C01E9E4	60	12/15/21	12/17/21
C01E956	38	12/15/21	12/17/21
C01E99D	44	12/15/21	12/17/21
C01E9CF	57	12/15/21	6/5/22
C01E998	22	12/15/21	12/17/21
C01E984	50	12/15/21	12/17/21
C01E93C	52	12/15/21	6/5/22
C01E9EC	24	12/15/21	6/5/22
C01E9B8	54	12/15/21	6/5/22
C01EA00	53	12/16/21	12/17/21
C01E983	39	12/16/21	12/17/21
C01E984	53	3/5/22	3/8/22
C01E982	55	3/5/22	3/13/22

Tag	Collar ID	Date Collar Deployed	Date Collar Removed
C01E982	55	3/5/22	6/5/22
C01E888	22	3/5/22	3/6/22
C01E983	34	3/6/22	6/5/22
C01E982	57	3/13/22	3/26/22
C01E982	55	5/17/22	3/13/22
C01E982	55	5/17/22	6/5/22
C01E984	61	5/17/22	5/23/22
C01E8D6	39	5/17/22	6/5/22
C01E943	40	5/17/22	6/2/22
C01E947	22	5/17/22	6/5/22
C01E8BB	36	5/17/22	6/5/22

Chapter 3 Environmental and Social Influences on Affiliative Behavior

3.1 Abstract

The evolution and maintenance of monogamous behavior, and affiliative behavior more broadly, has been a longstanding interest of behavioral and evolutionary biologists. Researchers have focused on understanding the mutual benefits for individuals to affiliate and how they outweigh potential costs of grouping behavior and/or social fidelity. Vole (*Microtus*) species have been models for studies on the evolution and maintenance of affiliative behavior, as researchers in a variety of disciplines have used behavioral, physiological, and neuroscience studies to characterize the differences among species in the wild and the laboratory. In prairie voles (*Microtus ochrogaster*), the formation of communal groups consisting of multiple unrelated adults has been observed most commonly in the autumn and winter months. However, it is not clear what drives these seasonal patterns in affiliative behavior. Here, we compared three existing, non-mutually exclusive hypotheses to explain increased affiliative behavior, as a means to determine whether social variables, abiotic environmental factors, and/or individual traits most reliably predict close social contact in prairie voles. We estimated social contacts of voles by characterizing their social networks using data collected from traditional live trapping methods and automated passive methods (radio-frequency identification), and summarized the number (unweighted degree) and strength (degree weight) of recorded contacts through social network analysis. We used these measures as our response variables with three sets of predictor variables—social, abiotic environmental, and individual factors. From the results of our selected

models, we found that social variables were most strongly associated with unweighted degree and degree weight. Specifically, adult sex ratio negatively predicted the number of connections per individual and had a nonlinear relationship with connection strength, such that connection strength was highest when sex ratio was female- or male-biased. Adult population density also had a nonlinear relationship with connection strength, such that connection strength was highest at both low and high extremes of population density. Together, these results suggest that demographic features of a population, rather than seasonal features of the environment, drive patterns of affiliative behavior in prairie voles. Our study helps to explain the variation recorded over the last 45+ years of studies in prairie voles, as demographic features such as sex ratio may be more dynamic and less predictable than seasonal changes such as daylight hours.

3.2 Introduction

All animals engage in some type of social interaction with conspecifics, whether it be competing for resources and mates, avoiding conflict, affiliating, or cooperating. Even solitary and non-social species use social signals to avoid risky or costly confrontations. For example, North American red squirrels (*Tamiasciurus hudsonicus*) use vocal signals to ward off resource competitors and adjust this signaling behavior based on their familiarity with conspecific neighbors (Siracusa et al., 2017, 2019). At the most basic level, social behaviors may consist of proactive tendencies to approach conspecifics or a lack of avoidance behaviors, while the most complex social behaviors include reconciliation, empathy, and coordinated cooperation (Raulo & Dantzer, 2018). These more complex social behaviors are predicted to evolve only when the fitness costs of affiliation and group behavior are outweighed by their fitness benefits.

The costs of group living, including increased potential for disease transmission and increased competition for local resources, may be overcome by benefits such as reduced

predation risk, more efficient thermoregulation, shared information, and increased offspring survival (Krause & Ruxton, 2002). While close contact, helping behavior, and group living can be enforced with coercion and aggression by dominant individuals, as has been observed in meerkats (*Suricata suricatta*; Clutton-Brock et al., 2006), social species may also be motivated and rewarded by affiliative behavior (Carter, 1998). For pair- and group-living species, affiliative behaviors such as huddling and grooming may help group members maintain homeostasis and regulate physiological stress (e.g., Feh & Mazières, 1993; Hennessey et al., 2008), potentially offering benefits for health (Uchino, 2006) and wellbeing (*sensu*: Ryff & Singer, 1998), in addition to immediate survival and reproductive benefits. For example, the presence of affiliative social partners has been linked to lower blood pressure, lower incidence of heart disease, and better immune function (reviewed in Uchino, 2006).

Since the 1980s, voles (*Microtus* spp.) have been valuable rodent models for understanding the benefits of affiliative behavior. *Microtus* is one of the most diverse mammalian genera, with an evolutionary history of rapid speciation (Barbosa et al., 2018). As such, closely related species of voles have divergent social and mating systems, lending themselves to comparative studies of affiliative behavior. Prairie voles (*Microtus ochrogaster*) are a socially monogamous species that readily forms lasting and selective pair bonds through cohabitation or mating (reviewed in Carter & Getz, 1993). The behavior, physiology, and neurobiology of prairie voles have been extensively compared to that of two polygynous species: montane voles (*Microtus montanus*) and meadow voles (*Microtus pennsylvanicus*), which mate promiscuously and do not form pair bonds (Madison, 1980a; Shapiro & Dewsbury, 1990). Interestingly, both montane voles and meadow voles are solitary but may form extended family

groups under high density (Jannett, 1978) or low temperature (Madison, et al., 1984) conditions, respectively.

A prairie vole will form a selective social preference for another individual after mating with them, or within 24 hours of cohabitation (Cho et al., 1999; Insel & Hulihan, 1995; Insel et al., 1995). In the laboratory, prairie voles spend significantly more time huddling or in side-by-side contact than polygynous montane voles (Shapiro & Dewsbury, 1990). Prairie voles also display higher levels of maternal (McGuire & Novak, 1984) and paternal (Oliveras & Novak, 1986) care of offspring than polygynous meadow voles. Historically, differences in affiliative behavior between monogamous and polygynous vole species have been linked to divergent density and distribution of oxytocin receptors (Insel & Shapiro, 1992; reviewed in Insel, 1992) and vasopressin receptor expression (Hammock & Young, 2004; Lim et al., 2004; Young et al., 1999) in the brain, although recent advances indicate that more complex neurobiological mechanisms mediate pair bonding (Berendzen et al., 2023; Goodson et al., 2005; O'Connell et al., 2012).

In addition to the variation that exists *among* species, there can be *within-species* behavioral variation from one time point to another. One pronounced example of this is the seasonal variation in meadow vole social behavior. Meadow voles, montane voles, and prairie voles all live in temperate North America, where they experience seasonal variation in weather patterns. During the summer months, meadow voles are solitary, with females defending exclusive breeding territories and males mating opportunistically (Boonstra et al., 1993; Madison, 1980b). However, during the winter and spring months, this territoriality breaks down and females begin to show social tolerance and enhanced preference for conspecifics, resulting in loose groups of unrelated individuals in shared nests (Madison et al., 1984; Madison & McShea,

1987). In the laboratory, this is marked by reduced aggression and increased huddling between females (Ondrasek et al., 2015). Changes in behavior and physiology can be induced in laboratory-bred voles maintained under short day (10 daylight hours) conditions (Dark & Zucker, 1983; Ondrasek et al., 2015), although reduced temperature and food availability can also increase social tolerance and affiliative behavior (Ondrasek et al., 2015).

Huddling and nest sharing provide thermoregulatory benefits for voles and other small mammals through energy and water conservation (Madison, 1984). As such, increased affiliative and group-living behavior in meadow voles may have evolved as a thermoregulatory mechanism because it increases overwintering survival (Madison, 1984; but see Berteaux et al., 1996). However, additional factors are known to influence affiliative behavior, such as high population density (e.g., family group formation in montane voles; Jannett, 1978) and high predation risk (e.g., communal nesting in taiga voles, *Microtus xanthognathus*; Wolff & Lidicker, 1981). Adult sex ratio may also affect mating behavior and the degree of monogamy exhibited in a population. For example, evolutionary theory predicts that males should increase mate guarding and monogamous behavior when the number of males in a population is larger than the number of females (i.e., there is a male-biased sex ratio; Emlen & Oring, 1977; Shuster & Wade, 2003).

While populations of wild prairie voles exhibit affiliative behavior and may maintain extended family groups throughout the year, they also form communal groups of unrelated conspecifics that are more common in the autumn and winter months (Getz et al., 1993). However, despite sharing a geographical range with meadow voles and experiencing pronounced population cycles (Getz et al., 1987), past research has not linked communal group formation or periods of increased affiliative behavior explicitly to cold temperatures or high population density (Getz et al., 1992, 1993; Getz and McGuire, 1997). Studies of natal philopatry in semi-

natural enclosures have observed a positive relationship between the number of philopatric groups and population density over the course of a summer season (Cochran & Solomon, 2000; Lucia et al., 2008), suggesting that population density may drive the formation of extended family groups, as in montane voles (Jannett, 1978). However, these studies measured only the formation of groups due to delayed dispersal of natal offspring, rather than the addition of any unrelated individuals, and took place only over the summer months. Therefore, further research is necessary to understand any changes in social dynamics over longer periods of time and across varied conditions.

In addition, researchers have speculated that communal groups of prairie voles form when rates of nest predation by snakes are low, due to the timing in patterns of juvenile mortality and a previous snake exclusion study (Getz et al., 1990). The snake exclusion study used an enclosure designed to allow the movement of voles and mammals over and through the walls, while impeding the entry of snakes into the enclosures (Getz et al., 1990). They found that juvenile mortality, but not adult mortality, was reduced in the snake exclusion zone—and that this reduction in juvenile mortality was associated with formation of communal social groups (Getz et al., 1990). This particular paper did not offer a mechanistic explanation for the formation of the communal groups and their relationship to juvenile mortality, nor the addition of unrelated adults to the philopatric groups, but this study has persisted as an explanation for the formation of communal groups in the years since (e.g., Getz et al., 1993, 2003; Getz & Carter, 1996; Solomon & Crist, 2008; Lucia & Keane, 2011), often with the suggestion that increased juvenile survival leads to higher levels of natal philopatry. However, socioecological theory predicts the opposite pattern: that social group formation, philopatry, and cooperative breeding should increase with predation risk (Krause & Ruxton, 2002), as evidenced in studies of

freshwater fish (Groenewoud et al., 2016; Kelley et al., 2011), Siberian jays (*Perisoreus infaustus*; Griesser et al., 2006), and cercopithecoid primates (Hill & Lee, 1998), for example. Therefore, the assumption that communal group formation is associated with low juvenile mortality (and/or low predation risk) necessitates confirmation, with a higher level of explanatory detail, or re-evaluation.

Importantly, during the onset of autumn in the temperate areas of North America where they live, voles likely experience cold temperatures, maximum population density, the cessation of snake predation (as snakes are ectothermic), and the end of their peak breeding season (i.e., fewer new juveniles) simultaneously. As such, the multiple seasonal factors that may be associated with periods of high affiliative behavior between prairie voles have not been systematically compared. In this study, we aimed to test the hypotheses suggested by the previous literature about the causes of affiliative behavior and group living in prairie voles, using three years of data from populations of prairie voles in semi-natural outdoor field enclosures. We compared three existing hypotheses to explain seasonal variation in prairie vole affiliative behavior, as a means to narrow down the strongest potential influences on close social contact behavior, with the recognition that these hypotheses are not mutually exclusive. Because the social contact data used in this study relies on spatial-temporal overlap of individuals, rather than shared nest usage or direct observation of social groups, we focused on the seasonal and environmental influences of generalized social contact data, rather than group formation.

Specifically, we evaluated the hypotheses that 1) aspects of the social environment (specifically, high population density, male-biased sex ratio, and low juvenile mortality) are most strongly associated with close social contact; 2) abiotic aspects of the physical environment (specifically, short days, low temperature, and low rainfall) are most strongly associated with

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close social contact; and 3) physical aspects of the individual animal (specifically, sex, age, and body weight) are most strongly associated with close social contact.

We include this latter "individual traits hypothesis" to account for the possibility that differences in social behavior are related to sex, body condition, or age, rather than season. Prairie voles demonstrate sex differences in the development of pair bonds and social motivation (e.g., Brusman et al., 2022), and previous research has found evidence for changes in social behavior over the lifespan, consistent with shifts in life history trajectory (Kelly et al., 2018; Powell et al., 2022). We also consider the possibility that a combination of social, abiotic, and individual effects would explain variation in social behavior, rather than any single predominant set of these factors.

Based on previous research described above, we hypothesized that social factors would be more strongly associated with aspects of affiliative behavior among prairie voles, in contrast to the primarily temperature-related affiliation observed in meadow voles (Dark & Zucker, 1983; Madison et al., 1984; Madison & McShea, 1987; Ondrasek et al., 2015). This would be demonstrated by the selection of our social model as the best fit for our data, beyond our abiotic environmental model, individual model, a full model (that contains a combination of these factors), or a null model. Specifically, we predicted that high juvenile mortality would predict fewer unique social associations among individuals, and that these associations would be weak, with the understanding that this result would both confirm prior hypotheses from studies of wild prairie voles (e.g., Getz et al., 1990) *and* indicate an atypical relationship between juvenile predation and sociability. Conversely, a result that failed to support this prediction would indicate that the explanation for prairie vole communal groups should be reworked—either

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because patterns of juvenile mortality are not associated with close social contact or because other hypotheses are better-fit to explain close social contact.

3.3 Methods

3.3.1 Study Area

We conducted all fieldwork for the study at the Miami University Ecology Research Center (ERC) in Oxford, Ohio (39° 53'N, 84° 73'W), over 36 months (Jun 2019–Jun 2022). Vole populations were established in four 0.1 ha (32m x 32m) semi-natural outdoor enclosures, surrounded by 20-gauge galvanized steel walls. The walls of each enclosure were 75 cm high and extended 45 cm below-ground to prevent voles from digging out of the enclosure (Cochran and Solomon, 2000). An electrified wire ran along the top of the enclosure walls to exclude terrestrial predators from the study area; however, voles could experience natural predation by birds or snakes, as the enclosures were uncovered. We regularly checked the condition of the walls for holes and mowed a ~1-m strip at the inner edge of each enclosure to discourage digging near the walls.

Vegetation in all enclosures was mainly a mix of goldenrod (*Solidago* spp.), clover (*Trifolium* spp.), timothy (*Phleum* spp.), ryegrass (*Elymus* spp.), fescue (*Festuca* spp.), bluegrass (*Poa pratensis*), and ragweed (*Ambrosia* spp.), which provided natural food and cover for voles (Solomon et al., 2009). We surveyed the vegetation of our enclosures in June–July 2021, and we removed any woody and thorny plants such as brambles (*Rubus* spp.) and common teasel (*Dipsacus fullonum*) observed over the course study—these plants made up less than 15% of vegetation (**Supplementary Table 2.1**). Prior to the release of animals, all enclosures were verified to be empty by live trapping until each had been trapped for three consecutive days without catching any animals. We occasionally caught deer mice (*Peromyscus* spp.; N = 16) or

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short-tailed shrews (*Blarina brevicauda*; N = 4) during our data collection period, and they were released immediately outside of the enclosure, without handling.

3.3.2 Study Animals

In early June 2019, we released 48 adult (82 - 155 days old), laboratory-bred prairie voles, descended from voles originally captured in Illinois and bred by the Angela Grippo Laboratory at Northern Illinois University. We distributed voles such that each of four enclosures had founding populations of six males and six females, equivalent to a medium density natural population of 120 voles/ha (based upon density measurements in Getz et al., 1993, 2001). In late November 2019, we released an additional 28 adult (56 - 230 days old) laboratory-bred voles into the enclosures, to boost densities and genetic diversity entering the winter. Because we had 28 voles in this new cohort to split between four enclosures, we released seven voles into each enclosure, distributed as either 1) three females and four males or 2) four females and three males, based on the sex ratios in the enclosures at the time. This additional group of founding voles consisted of laboratory-bred adult prairie voles, also descended from voles originally captured in Illinois, bred by the Alex Ophir Lab at Cornell University. We did not house, transport, or release opposite-sex siblings into the same enclosure, to avoid inbreeding.

All founder voles were marked with passive integrated transponder (PIT) tags (Biomark: Boise, Idaho; 12mm HPT tags), inserted under the skin above the shoulder blades, prior to release, which enabled us to uniquely identify voles for the course of the study. Voles were allowed to breed naturally, and offspring were PIT tagged when they reached a weight large enough to safely tag (body mass of ~12 g, measured using a spring scale; MD personal observation). Voles were moved among populations in July 2019, August 2019, May 2021, July 2021, September 2021, and May 2022 to reduce possible inbreeding and attempt to equalize sex

ratios across the enclosures. To accomplish this, we censused all populations with intensive trapping over two weeks, selected subadults of the correct sex to move (using trapping records of sex by genital inspection), and relocated selected individuals to the center of their new enclosure for release (immediately after capture). Animals did not receive any supplemental feeding aside from the cracked corn used to bait traps, which is a low-nutrient food source that should not add caloric value to their diet (Desy & Batzli, 1989). All populations were maintained continuously in the outdoor enclosures for the course of the study, from June 2019 until they were removed and humanely euthanized in June 2022. From March 8 to July 1, 2020, we were not able to trap or perform population censuses, due to restrictions on research and travel set by the University of Michigan due to the COVID-19 pandemic.

3.3.3 Live Trapping

We used live trapping (hereafter, "trapping") during this study to track body condition and female reproductive condition, record social interaction data, and capture offspring for PIT tagging. We set up traps on a 5 m x 5 m grid in each enclosure, with three multiple-capture Ugglan traps (Granhab: Gnosjö, Sweden) at each grid location (**Figure 3.1**). Therefore, during each trapping session, approximately 75 traps were set in each enclosure. For each capture, we recorded individual identity by scanning the PIT tag with a handheld transponder, capture location as the trapping stake on the 5 x 5 grid (**Figure 3.1**), sex by genital inspection, body weight (g) to the nearest 0.5 g using a spring scale (Pesola: Schindellegi, Switzerland), and the PIT tag IDs of each other animal captured in the same trap, also scanned with a handheld transponder.

We trapped voles in each enclosure at least once per week (Sun–Sat) during the peak breeding seasons (May–Sep; with the exception of May–Jul 2020, when research and travel were restricted due to the COVID-19 pandemic) to ensure continuous data collection and to catch as many offspring as possible, while minimizing disturbance to populations. During the peak breeding season, we conducted either evening sessions (traps set and checked from 1700 to 2000 h) or overnight sessions (traps set and checked from 2000 to 600 h), depending on ambient temperature and the type of data we needed to collect. This trapping procedure reduced the amount of time that animals spent in traps and the amount of time that offspring were potentially left without a parent, while also taking advantage of the time period when prairie voles are most active (1800–2000 h; Sabol et al., 2018). In addition, we did not trap during heavy rain or thunderstorms and rescheduled missed trapping sessions later in the week when possible.

During late autumn–early spring (Oct–Apr), we trapped at least once every 30 days to monitor populations with minimal disturbance to animals during the colder winter months. Monthly trapping has previously been validated as an effective time interval for monitoring population density, survival, and sex ratio; in a previous study, calculations of these three variables through monthly trapping sessions closely followed the patterns shown by 3.5-day trapping intervals (Getz et al., 2006). This previous study determined that the resources required for more frequent trapping for monitoring population density, survival, and sex ratio do not significantly increase the amount of demographic information recorded (Getz et al., 2006). When the ambient temperature dropped below 5 °C overnight, we trapped during the day (approximately 800–1800 h) and checked traps every 1–2 hours, to avoid and mitigate animal exposure to cold temperatures.

3.3.4 Radio Frequency Identification (RFID) Data

We used a central RFID Reader (Biomark: Boise, Idaho; RM310/SM303 System) connected to 12 antennas dispersed throughout the enclosure on one of two 3 x 4 arrays, with antennas at a distance of either 8 or 7.33 m from each other (**Figure 3.1**), which detected PIT tags of any vole within 1-3 cm of the antenna surface (MD, personal observation) once per second (Sabol et al., 2018). Due to signal collision, the RFID antennas cannot record more than one PIT tag at a time (MD, personal observation; Biomark: Boise, Idaho, personal communications); therefore, social contact data were inferred from the lag time between the consecutive detection of two different PIT tags at the same antenna.

We only had one RFID system, so it was rotated among enclosures throughout the study period. The antennas were also rotated through the two different array configurations (**Figure 3.1**) to measure activity throughout the enclosure without creating "dead zones" of undetected habitat area, using methods established and described in Sabol et al. (2018). From June to August 2019, the RFID system was rotated through the two array configurations every 2 days, and between enclosures every 4–8 days, for an experiment not described here. From September 2019 to June 2022, the RFID system was stationary in one of two enclosures, to collect long-term social network data on a single population. However, from April 2020 to July 2021, the RFID reader was not functional, so we do not have RFID data for that period. See **Supplementary Table 3.1** for full details of the RFID array rotation.

3.3.5 Construction of Social Networks

To obtain as comprehensive a picture as possible of the social structures and relationships present in our prairie vole populations, we compiled all trapping and RFID data into a single social network. All analyses were performed in R (version 3.6.1); social networks were

constructed using the R packages asnipe (version 1.1.16; Farine, 2017), timeordered (version

0.9.9; Blonder & Dornhaus, 2011), and igraph (version 1.5.1; Csardi & Nepusz, 2006). Using

methods developed by Solomon et al. (2009), we used spatial-temporal co-occurrences of

animals at the same trapping location (i.e., trapping stake - details in *3.3.3* Live Trapping) or in

the same trap during a trapping session as social contacts (i.e., social network edges), weighted

by the social ratio index (SRI), which is the proportion of the number of times that a pair was

recorded together to the total number of times each individual was recorded (Cairns & Schwager,

1987). For example, if two individuals were caught in the same trap together, or at the same

trapping location at the same time (i.e., spatial-temporal co-occurrence), they were considered to

be associating. This observed association would then be weighted by the number of times each

individual was trapped (either alone, or with a different social partner of either sex) and the

number of times both individuals were trapped during the *same* trapping session at *separate*

locations (i.e., spatial but not temporal co-occurrence).

      We ran the raw RFID data, consisting of a continuous temporal stream of individual

detections, through a Gaussian mixture model (GMM), which used antenna location and time in

seconds from the start of the recording period to create grouping events based on spatial and

temporal overlap (Psorakis et al., 2012). The output data are organized into a matrix of *K* groups

x *N* individuals, where individuals placed by the model into the same group (range: 6–81551

seconds; median: 2686 seconds; mean: 8989 seconds) have pairwise social contacts, also

weighted by their SRI (Cairns & Schwager, 1987; Farine, 2013). For example, if two individuals

were detected by the same RFID antenna 300 seconds (i.e., 5 min) apart, they would be assigned

a social contact, which would then by weighted by the number of times that same pair of

individuals was observed together in separate instances, the number of times each individual had

been detected by itself or with another partner of either sex, and the number of times that both individuals had been observed at the same time but not together (Hoppitt and Farine, 2018). Although the range of lag times between detections considered to be social contacts by our GMM is large, with high variability in the data, we did not threshold the data at a certain point (e.g., remove all estimated social contacts with lag times longer than 10 minutes between the detection of two individuals). The benefit of using a GMM for detecting likely social contacts is that researchers do not have to set an arbitrary time threshold or cutoff—the algorithm determines this cutoff through permutations of the raw data stream (Psorakis et al., 2012, 2015). See **Figure 2.5** for the full distribution of these data.

We converted the time units for both trapping (days) and RFID (seconds) data into a continuous Julian date scale (i.e., 1–365 for 2019, 366–720 for 2020, etc.). This allowed us to compile both types of data into a single temporal stream, with a signifier for the data collection method (trapping vs RFID). We used this compilation of social contact data to construct a time-ordered network (**Figure 3.2**), which allows for the analysis and modeling of social network dynamics, or how the structure of a network changes over time (Blonder & Dornhaus, 2011). In this construct, each individual is assigned a node at each time point during which they were observed, which is connected horizontally (within a single time point) to other nodes (i.e., individuals) and vertically to the same node at different time points. Therefore, horizontal connections between nodes (hereafter, "edges") represent individual social contacts. Because we were interested in seasonal aspects of social affiliation, we then constructed time-aggregated networks for each season (N = 6 seasons) by accumulating social interactions within set time periods (**Figure 3.3**). We divided seasons into late autumn–early spring (Oct 16–Mar 15) and late spring–early autumn (Mar 16–Oct 15), following the methods of Getz and McGuire (1997).

We chose to use these methods because Getz and McGuire (1997) focused on the formation of communal groups in the autumn and followed the expected timeline of juvenile mortality patterns due to snake predation. Because we were testing multiple hypotheses regarding seasonal changes in social behavior, these time periods were ecologically relevant for our questions and consistent with prior publications.

In each aggregated network, the number of unique edges that each individual has is known as the unweighted degree, and each edge is weighted by adding the weights of the time-ordered edges between two individuals within the aggregated time period. Simply put, the unweighted degree represents the number of individuals that the focal individual is associated with, and the degree weight represents the strength of that association. We used the unweighted degree and degree weight for each individual during each time period as the response variables for the remainder of our statistical analyses.

3.3.6 Predictor Variable Data Collection

3.3.6.1 Social Variables

We considered adult population density, adult sex ratio, and juvenile mortality to be features of the social environment, as they are determined by and relate to conspecifics in the population. Density and sex ratio are calculated using adults (classified by a body weight of at least 30 g; e.g., McGuire et al., 1989), as is the convention in prairie vole field studies, because adults define breeding units and are thought to be most influential on social organization (Getz et al., 1987). We used RFID and trapping data to estimate adult population density (hereafter, "population density") using—any adult detected during the relevant time frame was included. We calculated adult sex ratio (hereafter, "sex ratio") over the same time periods as the ratio of

adult males to all adult voles, or the proportion of males in the population. Finally, we estimated

juvenile mortality by recording the number of offspring trapped during each season who did *not*

survive until adulthood, measured as the number of offspring who were trapped at least once at a

juvenile weight under 20 g, but never trapped at an adult weight of 30 g. We used survival to

adulthood, rather than survival to juvenile age (as in Getz et al., 1990), to avoid estimating the

litter size of each mother, as absolute litter size can vary (e.g., 1 - 7 embryos; Ophir et al., 2018).

This measurement is also common in life history research, as survival to reproductive maturity is

an important factor in lifetime reproductive fitness (Stearns, 1976).

3.3.6.2 Abiotic Environmental Variables

We considered total cumulative rainfall (hereafter, "rainfall"), average number of

daylight hours per day (hereafter, "day length"), and average estimated soil temperature

(hereafter, "temperature") to be abiotic factors of the environment that may affect affiliative

behavior, based on previous studies (Dark & Zucker, 1983; Madison et al., 1984; Madison &

McShea, 1987; Ondrasek et al., 2015). Each of these variables was measured daily and compiled

as one value per season, assigned to all individuals.

Researchers at the Miami University ERC (along with EPA Clean Air Status and Trends

Network [CASTNET], EPA National Atmospheric Deposition Program [NADP], and Ohio

Agricultural Research and Development Center [OARDC]) maintain a weather station at the

ERC (established in 1982), adjacent to our animal enclosures, that records air temperature, solar

radiation, ozone, wind speed, wind direction, dry deposition on air filters, and precipitation,

along with conductivity, pH, and wet deposition of rain water samples. Historically, soil

temperature data were also collected at 5 cm and 10 cm belowground, but these data were only

collected from the start of data collection in 1982 until 2007.

We used the precipitation data recorded on-site at the ERC to calculate cumulative rainfall and the solar radiation data to calculate average day length. Because prairie voles are semi-fossorial, digging underground burrows with an average depth of 15-18 cm (Davis & Kalisz, 1992; Mankin & Getz, 1994), soil temperature may be more indicative of the realized temperature that voles experience than air temperature (Getz et al., 1993). Therefore, we used the historical soil and air temperature data collected by the ERC weather station from 1982 to 2007 to validate a model from Zheng et al. (1993), which estimates soil temperature at 10 cm depth from air temperature values. This method uses an 11-day running average of daily air temperature and a rate scalar determined by a regression model of temperature averages at six sites across the United States (Zheng et al., 1993). The observed soil temperature values from 1972 to 2007 had a Pearson correlation of 0.97 and an R^2 of 0.93 with the estimated soil temperature values that we calculated using air temperature values recorded during the same time period. Our recorded R^2 was within the range of R^2 calculated by Zheng et al. (1993), validating the efficacy of this method for our field site. Therefore, we used the same method to estimate soil temperature from air temperature values for the time frame of our study, when soil temperature was not directly recorded.

From June 27, 2019, to March 16, 2020, the sensors for precipitation and air temperature were non-functional. Additionally, from February 1, 2021, to June 6, 2022, the solar radiation sensor was non-functional. For these time periods, we used publicly available data from the closest NOAA Climate Data station, at the Hamilton Butler County Regional Airport (39.36°N, 84.52°W), approximately 30 km southeast of the ERC. We compared precipitation data from both sites, using records from October 1, 2021, to October 1, 2022, and found that daily cumulative precipitation at the ERC and the Hamilton Butler County Regional Airport were

significantly correlated ($p < 0.0001$; $R^2 = 0.36$). Daily air temperature readings from the ERC and Hamilton Butler County Regional Airport were also significantly correlated ($p < 0.0001$; R2 = 0.64), using data from October 1, 2021, to April 28, 2022.

3.3.6.3 Individual Variables

We used sex, body weight (g), and age class as individual features that could affect affiliative behavior. We recorded sex and body weight of each individual every time they were trapped and handled (details in **3.3.3** Live Trapping).

To reduce any physical or behavioral impact of handling, individual voles were not handled if they had already been trapped in the previous three days, so we did not have a weight associated with every trapping event. We used the median weight for each individual during each seasonal time period. The number of body weight measurements recorded for each individual ranged from 1–31 (mean = 5, median = 2), so the median measurement was used to account for individuals who had multiple weight recordings. Finally, we calculated the age class for each individual at the median date for each seasonal time period, based on weight. Males were classified as juveniles when under 20 g, subadults between 20–29 g, and adults when 30 g or above (McGuire et al., 1989). Females were classified as juveniles when under 20 g, subadults between 20– 27 g, and adults when 28 g or above (McGuire et al., 1989). Because there were very few juveniles (n = 7) in our final dataset with all other associated information, we included only subadults and adults in our final analyses.

3.3.7 Statistical Analyses to Model Associations with Seasonal Change in Affiliation

We performed two sets of model selection to determine the most reliable predictors (from the social, abiotic, and individual factors we measured) of two measures of affiliation: unweighted degree and degree weight. We centered and scaled all numeric predictor variables by

two standard deviations prior to model inclusion, to allow comparison between continuous and binary variables (Gelman, 2008).

3.3.7.1 Modeling Effects on Unweighted Degree

We used zero-inflated Poisson models to measure the associations between predictors and unweighted degree as a response variable, because degree is a count (i.e., degree = number of associations) and our data were zero-inflated, as determined through visual inspection of the data and testing with the R package glmmTMB (version 1.1.8; Brooks et al., 2017). Zero inflation occurs when there are more zeros in the count data than expected by chance, due to experimental design or some aspect of data structure. In our data, the structural zeros occurred because all individuals recorded alone (i.e., never with a partner) were assigned a degree of zero. We note that due to our methods, we were unable to differentiate between the possibilities that the focal individual was truly less social or that we just missed observing that individual's associations (i.e., they occurred outside of our recordings). All models included in the model selection are listed in **Table 3.1**.

When building our set of models to predict unweighted degree, we started with a null model consisting of only the response variable, the intercept, and the random effects of enclosure (i.e., nested population membership and vole individual identity; four levels) and data collection method (two levels), as trapping or the combination of trapping and RFID. These types of blocking effects are common in ecology and evolution models to adjust for pseudoreplication and control for variation due to confounding effects; however, a common rule of thumb restricts random effects to those with five or more levels (Bolker et al., 2009). More recent studies have systematically analyzed the use of random effects with fewer than five levels and provided guidelines to include categorical variables with few levels as random effects, as long as 1) the

researcher is not interested in interpreting the random effect and 2) the model does not become singularly fit (i.e., not generalizable; Gomes, 2022; Oberpriller et al., 2022). We aimed to include enclosure and method in our models to account for known effects of these variables, without interpreting those effects (i.e., control noise and confounding factors in our data), so we followed the more recent statistical guidelines from Gomes (2022) and Oberpriller et al. (2022).

We then added our social variables: population density, sex ratio, and estimated juvenile mortality (hereafter, "juvenile mortality"). We also included population density^2 and sex ratio^2 to account for non-linear effects of these factors on degree weight, using orthogonal polynomials to avoid collinearity between the linear and squared terms. We used the variance inflation factor (VIF) of the predictor variables to measure the severity of multicollinearity between predictors, with a cutoff of 3 (Zuur et al., 2010). Using this measure, we found that the population density term was severely multicollinear with the sex ratio term and juvenile mortality (max VIF = 25008). To correct this high VIF, we removed the population density term (including population density^2 as part of the orthogonal polynomial term) and placed it into its own alternative model. Therefore, we had one social model including the sex ratio term and juvenile mortality, along with a second social model including the population density term as the only predictor variable. Both of these models had VIF below our cutoff of 3; by adding both models to our model selection, we were able to determine which of our *a priori* predictor variables most reliably predicted our response variable, without the confounding effect of multicollinearity on our regression estimates.

Our next model built on the null model with the addition of abiotic environmental variables, including temperature, rainfall, and day length. Again, we found that two of our selected predictor variables were highly multicollinear (max VIF = 49.6), so we split rainfall and

day length into two separate models. This gave us one model with temperature and rainfall as predictor variables and a second model with temperature and day length as predictor variables. VIF of all variables was below our cutoff, so we did not alter the models further.

The next model investigated the effects of individual variables on unweighted degree by adding body weight, age class (binary: subadult or adult), and sex (binary: female or male) to our null model. We did not detect multicollinearity above 3 VIF in our individual model, so we did not change anything in this model to finalize it.

Our final model accounted for the possibility that a combination of social, abiotic, and individual factors might influence unweighted degree over and beyond the effects of any single set of these variables (our "full model"). Using Akaike Information Criterion corrected for small sample size (AICc), which is an information score that measures prediction error and accounts for both the number of predictors in a model and the sample size (Burnham & Anderson, 2002, 2004), we found that our social model including the combination of the sex ratio term and juvenile mortality was a better fit for our data than our social model including the population density term. A model was considered better fitting than another if its AICc was at least two units lower (i.e., ΔAICc $\leq$ -2). Therefore, we included the sex ratio term and juvenile mortality in our full model. Conversely, our competing abiotic environmental models did not differ in AICc, so we created competing full models to include rainfall and day length, respectively. Additionally, the sex ratio term was multicollinear with both rainfall and day length, so we split these into a "social full model" with our social variables and two "abiotic environmental full models" with our abiotic environmental variables.

Our social full model added the sex ratio term, juvenile mortality, temperature, body weight, age class (binary: subadult or adult), and sex (binary: female or male) to our null model.

Our first abiotic environmental full model included temperature, day length, body weight, age class (binary: subadult or adult), and sex (binary: female or male) as predictor variables. Our second abiotic environmental full model included temperature, rainfall, body weight, age class (binary: subadult or adult), and sex (binary: female or male) as predictor variables.

We selected the best-fitting model from these nine options (i.e., one null model, two social models, two environmental models, one individual model, and three full models) using AICc (**Table 3.1**), and then looked at the effect sizes of the predictor variables in the selected model to determine which factors most reliably predicted unweighted degree.

3.3.7.2 Modeling Effects on Degree Weight

We took the same analytical steps to narrow down effects of social, abiotic, and individual factors on degree weight. Here, we used linear mixed effects models conducted with the R packages lme4 (version 1.1-35.1; Bates et al., 2015) and lmerTest (version 3.1-3; Kuznetsova et al., 2017). Degree weight is calculated as the SRI, which is a proportion, so we performed an arcsine transformation prior to inclusion in our linear models (Sokal & Rohlf, 1995; Zar, 1998; Gotelli & Ellison 2004; but see Warton & Hui, 2011). We could not use a log or logit transformation of the proportions because they are not inclusive of zeros. As our degree (count) data were zero-inflated, each individual with a degree of zero also had a degree weight of zero. All models included in the model selection are listed in **Table 3.3**. Again, we started the model selection process with a null model consisting of only the response variable, the intercept, and the random effects of enclosure (i.e., nested population membership and individual identity; four levels) and data collection method (two levels), as trapping or the combination of trapping and RFID.

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From here, we added our social variables: population density, sex ratio, and juvenile mortality. We also included population density^2 and sex ratio^2 to account for non-linear effects of these factors on degree weight, using orthogonal polynomials to avoid collinearity between the linear and squared terms. Again, we used a VIF cutoff of 3 when measuring the severity of multicollinearity between predictors (Zuur et al., 2010). We corrected a high VIF by separating the polynomial population density term into an additional competing model without the polynomial sex ratio term and juvenile mortality, which were still grouped together in the original model.

We then added our abiotic environmental variables of temperature, rainfall, and day length to the null model. Rainfall and day length hours were multicollinear, so we separated them into competing models, which both included temperature.

Next, we created our model to measure the effects of individual variables on degree weight by adding body weight, age class (binary: subadult or adult), and sex (binary: female or male) to our null model. We did not detect multicollinearity above 3 VIF, so we did not adjust anything in our individual model of degree weight.

Our final model accounted for the possibility that degree weight is influenced by a combination of social, abiotic, and individual variables, rather than one set. Following the model selection steps described in *3.3.7.1 Modeling Effects on Unweighted Degree*, we created a set of alternative models to separate multicollinear combinations of variables and reduce VIF. These four models were as follows, with alternative variables indicated in italics: 1) a model including the *sex ratio term*, *juvenile mortality*, body weight, age class, and sex; 2) a model including the *population density term*, body weight, age class, and sex; 3) a model including *rainfall*,

temperature, body weight, age class, and sex; and 4) a model including *day length*, *temperature*, body weight, age class, and sex.

We performed model selection on the 10 models using AICc (**Table 3.3**), selecting the simplest model from the set of models within two AICc (i.e., equivalent fits to the dataset). We then analyzed the effect sizes of the predictor variables in the selected model to determine which factors most reliably predicted degree weight.

3.3.7.3 Post Hoc Analyses on Adult Population Density and Sex Ratio

Our planned analyses showed evidence of significant, non-linear (quadratic) relationships between degree weight and both sex ratio and population density (see ***3.4.2 Factors Associated with Degree Weight***). We were interested in any differences in unweighted degree or degree weight over time, whether opposite-sex or same-sex social contacts. However, upon seeing this result, we were interested in determining whether the observed quadratic relationship between our response variable and predictor variables was driven by a certain type of social contact. Changes in the weight and distribution of opposite-sex social contacts or same-sex social contacts may have different implications on the processes underlying dynamic social behavior. For example, opposite-sex social contacts may be indicative of mating behavior, while same-sex social contacts may be indicative of social behavior more generally.

Therefore, we separated our social contact data by opposite-sex and same-sex contacts and ran two additional linear mixed effects models to explore the relationships between degree weight and 1) sex ratio, 2) population density. We were specifically interested in determining whether one set of contacts had a significantly higher or more pronounced non-linear effect on degree weight. Having already performed our model selection, we ran these models with our

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predictor of interest (i.e., either sex ratio or population density) as the only predictor, along with the random effects of enclosure and data collection method.

3.3.8 Ethical Note

All research procedures and methods were approved by the Institutional Animal Care and Use Committees (IACUC) at University of Michigan (protocol #PRO00010760) and Miami University (protocol #979) and performed in accordance with the American Society of Mammalogists guidelines for the use of wild mammals in research (Sikes et al., 2016).

3.4 Results

3.4.1 Factors Associated with Unweighted Degree

The full model for unweighted degree including the social variables was a significantly better fit for our dataset than any of our other candidate models, as indicated by ΔAICc ranging from 6.3–53.3 (**Table 3.1**). We analyzed the results of this model further, to understand the effects of specific predictor variables on unweighted degree.

This full model contained predictor variables for sex ratio as an orthogonal polynomial, juvenile mortality, temperature, age class, sex, and body weight. Of these variables, sex ratio, juvenile mortality, sex, and age class reliably predicted unweighted degree, in order of effect size (**Figure 3.4**; **Table 3.2**). Specifically, adults had significantly higher average unweighted degree than subadults ($\beta = 0.29$; CI = [0.03, 0.56]; **Figure 3.5**) and males had significantly higher average unweighted degree than females ($\beta = 0.21$; CI = [0.07, 0.36]; **Figure 3.6**). Estimated juvenile mortality was significantly and positively predictive of unweighted degree ($\beta = 0.56$; CI = [0.27, 0.84]; **Figure 3.7**), such that individual unweighted degree was high at times when

estimated juvenile mortality was high. Finally, sex ratio was the most reliable predictor of unweighted degree (β = -5.71; CI = [-7.77, -3.65]; **Figure 3.10**); when the number of males outweighed the number of females in a population, unweighted degree tended to be low.

3.4.2 Factors Associated with Degree Weight

The social model including the population density term; the social model including the sex ratio term and juvenile mortality; and the full model including the population density term, age class, sex, and body weight were all equivalent models, as indicated by ΔAICc within 2 of each other (**Table 3.3**). These three models were also significantly better fit to our data than the other candidate models, which had ΔAICc values ranging from 5.4 to 11.0 (**Table 3.3**). We further evaluated the results of both social models. The full model with the population density term was 1.6 AICc higher than the social model of population density, indicating that the addition of the individual variables (age class, sex, and body weight) did not improve the model fit. Therefore, we did not further investigate the results of this model.

The selected social model had only one predictor variable: population density (including its orthogonal polynomial to the second degree). In this model, the quadratic term of population density significantly predicted degree weight, such that degree weight was highest when adult population density was either very high or very low (p = 0.002; z = 3.06; **Table 3.4**; **Figure 3.9**). The second social model was also considered because it fell within 2 AICc of the selected model; this model included fixed effects for sex ratio (including its orthogonal polynomial to the second degree), as well as juvenile mortality. In this model, the quadratic term of sex ratio significantly predicted degree weight, such that degree weight was also highest when sex ratio was unbalanced at either extreme (i.e., more females than males or more males than females) (p <

0.001; z = 3.69; **Table 3.4**; **Figure 3.10**). In this set of analyses, we used p-values instead of confidence intervals to indicate significance because the estimates and confidence intervals of polynomial terms should not be directly interpreted.

3.4.3 Post Hoc Exploratory Analyses of Adult Population Density and Sex Ratio

We did not find any significant differences between the effects of sex ratio (**Figure 3.11**) on opposite-sex or same-sex degree weight (summary stats). Both relationships were non-lineae (quadratic) and followed the same pattern, with increases in degree weight when adult sex ratio was female-biased (low sex ratio) or male-biased (high sex ratio).

3.5 Discussion

In this study, we compared three potential explanations for previously observed seasonal variation in close social contacts within prairie vole populations. Because prairie voles are socially monogamous and repeated social contact between individuals, measured by co-occurrence at trapping sites and RFID antennas has been biologically validated with partner preference tests to indicate affiliative behavior (Sabol et al., 2018), we expect that the social contacts we have measured here are affiliative. However, both of these methods rely on spatial-temporal overlap to estimate the likelihood of interaction, rather than direct affiliative behaviors.

We set out to determine the seasonal variables that affect patterns of affiliative behavior and communal grouping, but our results indicate that social behavior is driven by non-seasonal population demographics, rather than seasonal changes in weather or predation pressure. We failed to find support for the hypotheses that close contact behavior among individuals is driven by the onset of cold temperatures (as in meadow voles; Madison, 1984) or a release of predation pressure on juveniles in the autumn, as predicted by previous studies in prairie voles (Getz et al.,

1990). In particular, sex ratio most reliably predicted the number of unique social connections for each individual (**Figure 3.4**; **Figure 3.8**). Similarly, sex ratio and population density had equivalent, non-linear effects on the strength of those connections (**Figure 3.9**; **Figure 3.10**). Together, these results demonstrated that when the sex ratios in our populations were male-biased, individuals had fewer unique connections but the connections with the individuals that they *did associate* with were stronger.

This finding directly follows broader evolutionary theory regarding monogamy and mating tactics from a male perspective. As the sex ratio in a population becomes more male-biased, the availability of female mates decreases, theoretically causing males to move toward mate-guarding and monogamous behavior (Emlen & Oring, 1977; Shuster & Wade, 2003). Computational models support the prediction that mate-guarding and monogamous behavior are strongly favored when the sex ratio is male-biased and when partners are scarce (Gomes et al., 2018; Schacht & Bell, 2016). However, this is only the case when mate-guarding is effective and extra-pair copulations are low; the mating behavior of other individuals in the population, which may be more dynamic than population demographics, also affects the optimal behavioral phenotype (Gomes et al., 2018; Rice et al., 2018).

Although it is fairly straightforward that individuals in male-biased populations would have a limited number of social connections, with high strength, in a socially monogamous species, we would not expect to find equally strong connections in female-biased populations (as we did; **Figure 3.10**). From a male perspective, mate-guarding and monogamous behavior should decrease when the number of potential female mates is higher than the number of male competitors. These conditions theoretically create an optimal environment for promiscuity, wandering tactics, and extra-pair mating. There are a couple of existing hypotheses that could

explain this finding. First, it is important to note that the increase in social connection strength for female-biased populations coincided with an increased number of unique social connections under the same conditions—therefore, our results indicate a general increase in social behavior or a higher number of close mating partners. It is plausible that the high connection strength within female-biased populations is not an indication of increased monogamous behavior in the same way that it might be for a male-biased population with high connection strength but few unique connections.

That being said, evolutionary theory tends to underestimate the benefits of extra-pair mating or promiscuity for monogamous females. Evidence suggests that females benefit from promiscuous tactics when sex ratios are female-biased (Liker et al., 2014) and that female-female aggression and competition is more common in monogamous mammals than theory would predict (Stockley & Bro-Jørgensen, 2010). In addition, one study found that a higher percentage of prairie vole females in populations with female-biased sex ratios had litters with multiple males than females in male-biased populations, suggesting that mating with multiple males provides benefits for females, as increased fertilization success (Rice et al., 2022). If females play a substantial role in mate choice and exhibit flexible mating behavior in a similar manner to males (as reviewed in Shuster et al., 2019), our findings support the idea that *members of both sexes* are more actively mate-searching in female-biased populations—when males are opting for promiscuous tactics over mate-guarding and females are also reaping benefits from these additional mating opportunities. In this type of social environment, both males and females would likely have higher frequencies of interactions, just due to increased movement around the enclosures, combined with possibilities for mating and same-sex competition.

When we conducted post-hoc analyses to explore some of these possibilities further, we found that the relationships of sex ratio with same-sex connection strength and opposite-sex connection strength were not significantly different. Visually examining the data, it appeared that the curve for the opposite-sex edges was more exaggerated in male-biased populations than the curve for the same-sex edges (**Figure 3.11**), but any pattern of this nature was not statistically detectable. Therefore, our data provide more support for the idea that general sociality or movement patterns increase in populations when the sex ratio is female-biased, as opposed to any potential implications for female mate-guarding, female-female aggression, or extreme male promiscuity.

So far, we have explained potential causes for the relationship between sex ratio and close social contact; however, population density was an equally reliable predictor of connection strength among individuals. Understanding the bidirectional effects of population density on social behavior and vice versa has also been a lasting question in the fields of ecology and behavior, especially for small mammal researchers. Populations of small mammals undergo multiannual density cycles, with density peaks and troughs at both extremes, leading researchers to investigate the factors that cause and regulate these extreme density fluctuations.

One leading hypothesis is the polymorphic behavior hypothesis (Chitty, 1960, 1967), which posits that socially tolerant, high reproductive phenotypes are selected for during periods of low density, while aggressive, low reproductive phenotypes are favored during periods of high density. This hypothesis was rejected by studies that found variability in social tolerance to be influenced by environmental factors and maternal effects, rather than heritable genetic differences (Boonstra and Boag, 1987). However, early life experience and epigenetic mechanisms also were found to influence reproductive phenotypes at high and low densities

(Edwards et al., 2021)—in other words, the mechanisms suggested in the polymorphic behavior hypothesis could be mediated by epigenetic rather than heritable factors (see also: McAdam et al., 2014).

In light of this hypothesis, our observed, non-linear relationship between adult population density and connection strength would suggest that periods of high population density led to repeated interactions among conspecific neighbors at close spacing. Additionally, more of these encounters at high density could be antagonistic interactions. Conversely, at low population densities, individuals might be more tolerant, facilitating affiliative interactions between individuals. Importantly, we are unable to distinguish between these possibilities in this study because we did not directly observe the social contacts in the analysis—therefore, we cannot determine whether any contacts were affiliative, antagonistic, or just indicative of the likelihood of encounter (i.e., with RFID). We have reason to believe, based on previous research, that many of our recorded contacts are affiliative, as social network measures generated with RFID data are correlated with partner preference in partner preference tests (Sabol et al., 2018); however, we are unable to validate our recorded social contacts with the data from this study.

Even given these limitations, this is the first study, to our knowledge, that has directly tested or compared existing hypotheses regarding the explanation for patterns of close social contact or affiliative behavior in prairie voles. This study provides evidence that the processes behind peaks in prairie vole social behavior are not unique, and rather, follow the same patterns observed in other species of voles and mammals more generally, aligning with evolutionary and socioecological theory. Demographic variables such as sex ratio and population density are associated with close social contact throughout the year, as in montane voles (Jannett, 1978) and following predictions regarding the evolution of monogamy (e.g., Shuster & Wade, 2003). Our

results demonstrate that the absence of a reliable seasonal pattern, as well as the historical lack of reliable associations between group formation and temperature or day length in previous studies, are due to the overarching influence of demographic variables, rather than the presence of a novel, unknown mechanism. However, future research should clarify the directionality and codependence of relationships between population density and sex ratio through experimental design, as all of our work here has been correlational. Additionally, **Chapter 2** of this dissertation focuses on the limitations of the RFID and trapping methods used here, where we further discuss the possibilities for high-resolution data to investigate these types of questions in detail. Advances in technology and future research will add to the certainty of hypotheses and predictions relating to seasonal and demographic influences on social behavior.

3.6 Acknowledgements

3.7 References

Barbosa, S., Paupério, J., Pavlova, S. V., Alves, P. C., & Searle, J. B. (2018). The Microtus voles: Resolving the phylogeny of one of the most speciose mammalian genera using genomics. *Molecular Phylogenetics and Evolution, 125*, 85-92. https://doi.org/10.1016/j.ympev.2018.03.017.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Berendzen, K. M., Sharma, R., Mandujano, M. A., Wei, Y., Rogers, F. D., Simmons, T. C., Seelke, A. M. H., Bond, J. M., Larios, R., Goodwin, N. L., Sherman, M., Parthasarthy, S., Espineda, I., Knoedler, J. R., Beery, A., Bales, K. L., Shah, N. M., & Manoli D. S. (2023). Oxytocin receptor is not required for social attachment in prairie voles. *Neuron, 111*(6), P787-796.E4. https://doi.org/10.1016/j.neuron.2022.12.011.

Berteaux, D., Bergeron, J. M., Thomas, D. W., & Lapierre, H. (1996). Solitude versus gregariousness: do physical benefits drive the choice in overwintering meadow voles?. *Oikos*, 330-336. https://doi.org/10.2307/3546205.

Blonder, B., & Dornhaus, A. (2011). Time-ordered networks reveal limitations to information flow in ant colonies. *PloS one, 6*(5), e20298. https://doi.org/10.1371/journal.pone.0020298.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J. S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution, 24*(3), 127-135. https://doi.org/10.1016/j.tree.2008.10.008.

Boonstra, R., & Boag, P. T. (1987). A test of the Polymorphic behavior hypothesis: inheritance of life-history traits in meadow voles *Microtus pennsylvanicus*. *Evolution, 41*(5), 929-947. https://doi.org/10.2307/2409183.

Boonstra, R., Xia, X., & Pavone, L. (1993). Mating system of the meadow vole, *Microtus pennsylvanicus*. *Behavioral Ecology, 4*(1), 83-89. https://doi.org/10.1093/beheco/4.1.83.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal, 9*(2), 378–400. https://doi.org/10.32614/RJ-2017-066.

Brusman, L. E., Protter, D. S., Fultz, A. C., Paulson, M. U., Chapel, G. D., Elges, I. O., Cameron, R. T., Beery, A. K., & Donaldson, Z. R. (2022). Emergent intra-pair sex differences and organized behavior in pair bonded prairie voles (*Microtus ochrogaster*). *Genes, Brain and Behavior, 21*(3), e12786. https://doi.org/10.1111/gbb.12786.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn*. Springer, New York. ISBN: 0387953647.

Burnham, K. P., & Anderson, D. R. (2004). Multimodel inference: understanding AIC and BIC in model selection. *Sociological Methods & Research, 33*(2), 261-304. https://doi.org/10.1177/0049124104268644.

Cairns, S. J., & Schwager, S. J. (1987). A comparison of association indices. *Animal Behaviour, 35*(5), 1454-1469. https://doi.org/10.1016/S0003-3472(87)80018-0.

Carter, C. S., & Getz, L. L. (1993). Monogamy and the prairie vole. *Scientific American, 268*(6), 100-106. https://doi.org/10.1038/scientificamerican0693-100.

Chitty, D. (1960). Population processes in the vole and their relevance to general theory. Canadian Journal of Zoology, 38(1), 99-113. https://doi.org/10.1139/z60-011.

Chitty, D. (1967). What Regulates Bird Population. *Ecology 48*(4), 698-701. https://doi.org/10.2307/1936523

Cho, M. M., DeVries, A. C., Williams, J. R., & Carter, C. S. (1999). The effects of oxytocin and vasopressin on partner preferences in male and female prairie voles (*Microtus ochrogaster*). *Behavioral Neuroscience, 113*(5), 1071. https://doi.org/10.1037//0735-7044.113.5.1071.

Clutton-Brock, T. H., Hodge, S. J., Spong, G., Russell, A. F., Jordan, N. R., Bennett, N. C., Sharpe, L. L., & Manser, M. B. (2006). Intrasexual competition and sexual selection in cooperative mammals. *Nature, 444*(7122), 1065-1068. https://doi.org/10.1038/nature05386.

Cochran, G. R., & Solomon, N. G. (2000). Effects of food supplementation on the social organization of prairie voles (Microtus ochrogaster). *Journal of Mammalogy, 81*(3), 746-757. https://doi.org/10.1644/1545-1542(2000)081%3C0746:EOFSOT%3E2.3.CO;2.

Csardi, G., & Nepusz, T. (2006). The igraph software package for complex network research. *InterJournal, Complex Systems, 1695*(5), 1-9.

Dark, J., & Zucker, I. (1983). Short photoperiods reduce winter energy requirements of the meadow vole, *Microtus pennsylvanicus*. *Physiology & Behavior, 31*(5), 699-702. https://doi.org/10.1016/S0031-9384(83)80006-7.

Davis, W. H., & Kalisz, P. J. (1992). Burrow systems of the prairie vole, *Microtus ochrogaster*, in central Kentucky. *Journal of Mammalogy, 73*(3), 582-585. https://doi.org/10.2307/1382028.

Desy, E. A., & Batzli, G. O. (1989). Effects of food availability and predation on prairie vole demography: a field experiment. *Ecology, 70*(2), 411-421. https://doi.org/10.2307/1937546.

Edwards, P. D., Frenette-Ling, C., Palme, R., & Boonstra, R. (2021). A mechanism for population self-regulation: Social density suppresses GnRH expression and reduces reproductivity in voles. *Journal of Animal Ecology, 90*(4), 784-795. https://doi.org/10.1111/1365-2656.13430.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*, 215-223. https://doi.org/10.1126/science.327542.

Farine, D. R. (2013). Animal social network inference and permutations for ecologists in R using asnipe. Methods in Ecology and Evolution, 4(12), 1187-1194. https://doi.org/10.1111/2041-210X.12121.

Farine, D. R. (2017). A guide to null models for animal social network analysis. *Methods in Ecology and Evolution, 8*(10), 1309-1320. https://doi.org/10.1111/2041-210X.12772.

Feh, C., & De Mazières, J. (1993). Grooming at a preferred site reduces heart rate in horses. *Animal Behaviour, 46*(6), 1191-1194. https://doi.org/10.1006/anbe.1993.1309.

Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in medicine, 27*(15), 2865-2873. https://doi.org/10.1002/sim.3107.

Getz, L. L., Gudermuth, D. F., & Benson, S. M. (1992). Pattern of nest occupancy of the prairie vole *Microtus ochrogaster* in different habitats. *American Midland Naturalist*, 197-202. https://doi.org/10.2307/2426425.

Getz, L. L., & McGuire, B. (1997). Communal nesting in prairie voles (*Microtus ochrogaster*): formation, composition, and persistence of communal groups. *Canadian Journal of Zoology, 75*(4), 525-534. https://doi.org/10.1139/z97-065.

Getz, L. L., McGuire, B., & Carter, C. S. (2003). Social behavior, reproduction and demography of the prairie vole, Microtus ochrogaster. *Ethology Ecology & Evolution*, *15*(2), 105-118. https://doi.org/10.1080/08927014.2003.9522676.

Getz, L. L., McGuire, B., Pizzuto, T., Hofmann, J. E., & Frase, B. (1993). Social organization of the prairie vole (*Microtus ochrogaster*). *Journal of Mammalogy, 74*(1), 44–58. https://doi.org/10.2307/1381904.

Getz, L. L., Hofmann, J. E., & Carter, C. S. (1987). Mating system and population fluctuations of the prairie vole, *Microtus ochrogaster*. *American Zoologist, 27*(3), 909-920. https://doi.org/10.1093/icb/27.3.909.

Getz, L. L., Hofmann, J. E., McGuire, B., & Dolan III, T. W. (2001). Twenty-five years of population fluctuations of *Microtus ochrogaster* and *M. pennsylvanicus* in three habitats in east-central Illinois. *Journal of Mammalogy, 82*, 22-34. http://dx.doi.org/10.1093/jmammal/82.1.22.

Getz, L. L., Hofmann, J. E., Simms, L., & McGuire, B. (2006). Efficacy of monthly and semi-monthly trapping of small mammal populations. *The American Midland Naturalist, 155*(2), 422-427. https://doi.org/10.1674/0003-0031(2006)155[422:EOMAST]2.0.CO;2.

Getz, L. L., Solomon, N. G., & Pizzuto, T. M. (1990). The effects of predation of snakes on social organization of the prairie vole, Microtus ochrogaster. American Midland Naturalist, 365-371. https://doi.org/10.2307/2426564.

Gomes, D. G. E. (2022). Should I use fixed effects or random effects when I have fewer than five levels of a grouping factor in a mixed-effects model? *PeerJ 10*:e12794. https://doi.org/10.7717/peerj.12794.

Gomes, B. V., Guimarães, D. M., Szczupak, D., & Neves, K. (2018). Female dispersion and sex ratios interact in the evolution of mating behavior: a computational model. *Scientific Reports, 8*(1), 2467. https://doi.org/10.1038/s41598-018-20790-7.

Goodson, J. L. (2005). The vertebrate social behavior network: evolutionary themes and variations. *Hormones and Behavior, 48*(1), 11-22. https://doi.org/10.1016/j.yhbeh.2005.02.003.

Gotelli, N. J. & Ellison, A. M. (2004). *A primer of ecological statistics.* Sinauer Associates. Sunderland, Massachusetts, USA. ISBN: 9781605350646.

Griesser, M., Nystrand, M., & Ekman, J. (2006). Reduced mortality selects for family cohesion in a social species. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1596), 1881-1886. https://doi.org/10.1098/rspb.2006.3527.

Groenewoud, F., Frommen, J. G., Josi, D., Tanaka, H., Jungwirth, A., & Taborsky, M. (2016). Predation risk drives social complexity in cooperative breeders. *Proceedings of the National Academy of Sciences*, *113*(15), 4104-4109. https://doi.org/10.1073/pnas.1524178113.

Hammock, E. A., & Young, L. J. (2004). Functional microsatellite polymorphism associated with divergent social structure in vole species. *Molecular Biology and Evolution, 21*(6), 1057-1063. https://doi.org/10.1093/molbev/msh104.

Hennessy, M. B., Zate, R., & Maken, D. S. (2008). Social buffering of the cortisol response of adult female guinea pigs. Physiology & behavior, 93(4-5), 883-888. https://doi.org/10.1016/j.physbeh.2007.12.005.

Hill, R. A., & Lee, P. C. (1998). Predation risk as an influence on group size in cercopithecoid primates: implications for social structure. *Journal of Zoology, 245*(4), 447-456. https://doi.org/10.1017/S0952836998008085.

Hodges, K. E., Mech, S., & Wolff, J. O. (2002). Sex and the single vole: effects of social grouping on prairie vole reproductive success. *Ethology, 108*(10), 871-884. https://doi.org/10.1046/j.1439-0310.2002.00822.x.

Hoppitt, W. J., & Farine, D. R. (2018). Association indices for quantifying social relationships: how to deal with missing observations of individuals or groups. *Animal Behaviour, 136*, 227-238. https://doi.org/10.1016/j.anbehav.2017.08.029.

Insel, T. R. (1992). Oxytocin—a neuropeptide for affiliation: evidence from behavioral, receptor autoradiographic, and comparative studies. *Psychoneuroendocrinology, 17*(1), 3-35. https://doi.org/10.1016/0306-4530(92)90073-G.

Insel, T. R., & Hulihan, T. J. (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. Behavioral neuroscience, 109(4), 782. https://doi.org/10.1037/0735-7044.109.4.782.

Insel, T. R., Preston, S., & Winslow, J. T. (1995). Mating in the monogamous male: behavioral consequences. *Physiology & Behavior, 57*(4), 615-627. https://doi.org/10.1016/0031-9384(94)00362-9.

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proceedings of the National Academy of Sciences, 89*(13), 5981-5985. https://doi.org/10.1073/pnas.89.13.5981.

Jannett, F. J., Jr. (1978). The density dependent formation of extended maternal families of the montane vole, *Microtus montanus nanus. Behavioral Ecology and Sociobiology, 3*:245-263. https://doi.org/10.1007/BF00296312.

Kelley, J. L., Morrell, L. J., Inskip, C., Krause, J., & Croft, D. P. (2011). Predation risk shapes social networks in fission-fusion populations. *PloS one*, *6*(8), e24280. https://doi.org/10.1371/journal.pone.0024280.

Kelly, A. M., Saunders, A. G., & Ophir, A. G. (2018). Mechanistic substrates of a life history transition in male prairie voles: Developmental plasticity in affiliation and aggression corresponds to nonapeptide neuronal function. *Hormones and Behavior, 99*, 14-24. https://doi.org/10.1016/j.yhbeh.2018.01.006.

Krause, J., Krause, S., Arlinghaus, R., Psorakis, I., Roberts, S., & Rutz, C. (2013). Reality mining of animal social systems. *Trends in Ecology & Evolution*, *28*(9), 541-551. https://doi.org/10.1016/j.tree.2013.06.002.

Krause, J., & Ruxton, G. (2002). *Living in Groups.* Oxford University Press, USA. ISBN: 0198508174.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13.

Liker, A., Freckleton, R. P., & Székely, T. (2014). Divorce and infidelity are associated with skewed adult sex ratios in birds. *Current Biology, 24*(8), 880-884. http://dx.doi.org/10.1016/j.cub.2014.02.059.

Lim, M. M., Wang, Z., Olazábal, D. E., Ren, X., Terwilliger, E. F., & Young, L. J. (2004). Enhanced partner preference in a promiscuous species by manipulating the expression of a single gene. *Nature, 429*(6993), 754-757. https://doi.org/10.1038/nature02539.

Lucia, K. E., & Keane, B. (2012). A field test of the effects of familiarity and relatedness on social associations and reproduction in prairie voles. *Behavioral Ecology and Sociobiology*, *66*, 13-27. https://doi.org/10.1007/s00265-011-1247-6.

Lucia, K. E., Keane, B., Hayes, L. D., Lin, Y. K., Schaefer, R. L., & Solomon, N. G. (2008). Philopatry in prairie voles: an evaluation of the habitat saturation hypothesis. *Behavioral Ecology, 19*(4), 774-783. https://doi.org/10.1093/beheco/arn028.

Lüdecke, D. (2018). ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *Journal of Open Source Software, 3*(26), 772. https://doi.org/10.21105/joss.00772.

Madison, D. M. (1980a). An integrated view of the social biology of *Microtus pennsylvanicus*. *The Biologist, 62*, 20-33.

Madison, D. M. (1980b). Space use and social structure in meadow voles, *Microtus pennsylvanicus*. *Behavioral Ecology and Sociobiology, 7*, 65-71. https://doi.org/10.1007/BF00302520.

Madison, D. M. (1984). Group nesting and its ecological and evolutionary significance in overwintering microtine rodents. In: Merritt, J. F. (ed.), *Winter ecology of small mammals. Special Publication no. 10, Canergie Museum of Natural History*, pp. 285-291. ISBN-13: 978-0935868104.

Madison, D. M., FitzGerald, R. W., & McShea, W. J. (1984). Dynamics of social nesting in overwintering meadow voles (*Microtus pennsylvanicus*): possible consequences for population cycling. *Behavioral Ecology and Sociobiology, 15*, 9-17. https://doi.org/10.1007/BF00310209.

Madison, D. M., & McShea, W. J. (1987). Seasonal changes in reproductive tolerance, spacing, and social organization in meadow voles: a microtine model. *American Zoologist, 27*(3), 899-908. https://doi.org/10.1093/icb/27.3.899.

Mankin, P. C., & Getz, L. L. (1994). Burrow morphology as related to social organization of *Microtus ochrogaster. Journal of Mammalogy, 75*(2), 492-499. https://doi.org/10.2307/1382573.

McAdam, A. G., Garant, D., & Wilson, A. J. (2014). The effects of others' genes: maternal and other indirect genetic effects. *Quantitative Genetics in the Wild*, 84-103.

McGuire, B., Bemis, W. E., & Vermeylen, F. (2014). Parental behaviour of prairie voles (Microtus ochrogaster) and meadow voles (M. pennsylvanicus) in relation to sex of offspring. *Behaviour*, *151*(4), 535-553. https://doi.org/10.1163/1568539X-00003141.

McGuire, B., & Novak, M. (1984). A comparison of maternal behaviour in the meadow vole (*Microtus pennsylvanicus*), prairie vole (*M. ochrogaster*) and pine vole (*M. pinetorum*). *Animal Behaviour, 32*(4), 1132-1141. https://doi.org/10.1016/S0003-3472(84)80229-8.

McGuire, B., Pizzuto, T., & Getz, L. L. (1990). Potential for social interaction in a natural population of prairie voles (Microtus ochrogaster). *Canadian Journal of Zoology, 68*(2), 391-398. https://doi.org/10.1139/z90-056.

Nakagawa, S., Johnson, P. C., & Schielzeth, H. (2017). The coefficient of determination R^2 and intra-class correlation coefficient from generalized linear mixed-effects models revisited and expanded. *Journal of the Royal Society Interface, 14*(134), 20170213. https://doi.org/10.1098/rsif.2017.0213.

O'Connell, L. A., & Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science, 336*(6085). 1154-1157. https://doi.org/10.1126/science.1218889.

Oliveras, D., & Novak, M. (1986). A comparison of paternal behaviour in the meadow vole *Microtus pennsylvanicus*, the pine vole *M. pinetorum* and the prairie vole *M. ochrogaster*. *Animal behaviour, 34*(2), 519-526. https://doi.org/10.1016/S0003-3472(86)80120-8.

Ondrasek, N. R., Wade, A., Burkhard, T., Hsu, K., Nguyen, T., Post, J., & Zucker, I. (2015). Environmental modulation of same-sex affiliative behavior in female meadow voles (*Microtus pennsylvanicus*). *Physiology & Behavior, 140*, 118-126. https://doi.org/10.1016/j.physbeh.2014.12.021.

Oberpriller, J., de Souza Leite, M., & Pichler, M. (2022). Fixed or random? On the reliability of mixed-effects models for a small number of levels in grouping variables. *Ecology and Evolution, 12*(7), e9062. https://doi.org/10.1002/ece3.9062.

Ophir, A. G., Phelps, S. M., Sorin, A. B., & Wolff, J. O. (2008). Social but not genetic monogamy is associated with greater breeding success in prairie voles. *Animal Behaviour, 75*(3), 1143-1154. https://doi.org/10.1016/j.anbehav.2007.09.022.

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. *Oikos, 118*(12), 1883-1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x.

Powell, J. M., Garvin, M. M., Lee, N. S., & Kelly, A. M. (2022). Behavioral trajectories of aging prairie voles (*Microtus ochrogaster*): Adapting behavior to social context wanes with advanced age. *Plos One, 17*(11), e0276897.https://doi.org/10.1371/journal.pone.0276897.

Psorakis, I., Voelkl, B., Garroway, C. J., Radersma, R., Aplin, L. M., Crates, R. A., Culina, A., Farine, D. R., Firth, J. A., Hinde, C. A., Kidd, L. R., Milligan, N. D., Roberts, S. J., Verhelst, B., & Sheldon, B. C. (2015). Inferring social structure from temporal data.

Behavioral Ecology and Sociobiology, 69, 857-866. https://doi.org/10.1007/s00265-015-1906-0.

Psorakis, I., Roberts, S. J., Rezek, I., & Sheldon, B. C. (2012). Inferring social network structure in ecological systems from spatio-temporal data streams. *Journal of the Royal Society Interface, 9*(76), 3055-3066. https://doi.org/10.1098/rsif.2012.0223.

Rice, M. A., Restrepo, L. F., & Ophir, A. G. (2018). When to cheat: modeling dynamics of paternity and promiscuity in socially monogamous prairie voles (*Microtus ochrogaster*). *Frontiers in Ecology and Evolution, 6*, 141. https://doi.org/10.3389/fevo.2018.00141.

Rice, M. A., Galindez, S. M., Garner, J. T., & Ophir, A. G. (2022). Effects of social and environmental contexts on multi-male mating and mixed paternity in socially monogamous female prairie voles. *Royal Society Open Science, 9*(10), 220298. https://doi.org/10.1098/rsos.220298.

Ryff, C. D., & Singer, B. (1998). The contours of positive human health. *Psychological Inquiry, 9*(1), 1-28. https://doi.org/10.1207/s15327965pli0901_1.

Sabol, A. C., Solomon, N. G., & Dantzer, B. (2018). How to study socially monogamous behavior in secretive animals? Using social network analyses and automated tracking systems to study the social behavior of prairie voles. *Frontiers in Ecology and Evolution, 6*, 178. https://doi.org/10.3389/fevo.2018.00178.

Schacht, R., & Bell, A. V. (2016). The evolution of monogamy in response to partner scarcity. *Scientific reports, 6*(1), 32472. https://doi.org/10.1038/srep32472.

Shapiro, L. E., & Dewsbury, D. A. (1990). Differences in affiliative behavior, pair bonding, and vaginal cytology in two species of vole (*Microtus ochrogaster and M. montanus*). *Journal of Comparative Psychology, 104*(3), 268. https://doi.org/10.1037/0735-7036.104.3.268.

Shuster, S. M., & Wade, M. J. (2003). *Mating systems and mating strategies.* Princeton University Press. ISBN 0-691-04931-9.

Shuster, S. M., Willen, R. M., Keane, B., & Solomon, N. G. (2019). Alternative mating tactics in socially monogamous prairie voles, *Microtus ochrogaster. Frontiers in Ecology and Evolution, 7*, 7. https://doi.org/10.3389/fevo.2019.00007.

Sikes, R. S., & Animal Care and Use Committee of the American Society of Mammalogists. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy, 97*(3), 663-688. https://doi.org/10.1093/jmammal/gyw078.

Siracusa, E., Boutin, S., Humphries, M. M., Gorrell, J. C., Coltman, D. W., Dantzer, B., Lane, J. E., & McAdam, A. G. (2017). Familiarity with neighbours affects intrusion risk in territorial red squirrels. *Animal Behaviour, 133*, 11-20. https://doi.org/10.1016/j.anbehav.2017.08.024.

Siracusa, E. R., Wilson, D. R., Studd, E. K., Boutin, S., Humphries, M. M., Dantzer, B., Lane, J. E., & McAdam, A. G. (2019). North American red squirrels mitigate costs of territory defence through social plasticity. *Animal Behaviour, 151*, 29-42. https://doi.org/10.1016/j.anbehav.2019.02.014.

Sokal, R. R. & Rohlf, F. H. (1995). *Biometry: the principles and practice of statistics in biological research. Third edition.* W. H. Freeman. New York, New York, USA. ISBN-13: 978-0716724117.

Solomon, N. G., & Crist, T. O. (2008). Estimates of reproductive success for group-living prairie voles, Microtus ochrogaster, in high-density populations. *Animal Behaviour*, *76*(3), 881-892. https://doi.org/10.1016/j.anbehav.2008.01.028.

Solomon, N. G., Richmond, A. R., Harding, P. A., Fries, A., Jacquemin, S., Schaefer, R. L., Lucia, K.E., & Keane, B. (2009). Polymorphism at the avpr1a locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Molecular Ecology, 18*(22), 4680-4695. https://doi.org/10.1111/j.1365-294X.2009.04361.x.

Stearns, S. C. (1976). Life-history tactics: a review of the ideas. *The Quarterly Review of Biology, 51*(1), 3-47. https://doi.org/10.1086/409052.

Stockley, P., & Bro-Jørgensen, J. (2011). Female competition and its evolutionary consequences in mammals. *Biological Reviews, 86*(2), 341-366. https://doi.org/10.1111/j.1469-185X.2010.00149.x.

Uchino, B. N. (2006). Social support and health: a review of physiological processes potentially underlying links to disease outcomes. *Journal of Behavioral Medicine, 29*, 377-387. https://doi.org/10.1007/s10865-006-9056-5.

Warton, D. I., & Hui, F. K. (2011). The arcsine is asinine: the analysis of proportions in ecology. *Ecology, 92*(1), 3-10. https://doi.org/10.1890/10-0340.1.

Wolff, J. O., & Lidicker Jr, W. Z. (1981). Communal winter nesting and food sharing in taiga voles. *Behavioral Ecology and Sociobiology*, 237-240. https://doi.org/10.1007/BF00299877.

Young, L. J., Nilsen, R., Waymire, K. G., MacGregor, G. R., & Insel, T. R. (1999). Increased affiliative response to vasopressin in mice expressing the V1a receptor from a monogamous vole. *Nature, 400*(6746), 766-768. https://doi.org/10.1038/23475.

Zar, J. H. (1998). *Biostatistical analysis. Fourth edition.* Prentice Hall. Englewood Cliffs, New Jersey, USA. ISBN 13: 9780130815422.

Zheng, D., Hunt Jr, E. R., & Running, S. W. (1993). A daily soil temperature model based on air temperature and precipitation for continental applications. *Climate Research, 2*(3), 183-191. https://doi.org/10.3354/cr002183.

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution, 1*(1), 3-14. https://doi.org/10.1111/j.2041-210X.2009.00001.x.

3.8 Tables and Figures

Table 3.1: Candidate zero inflated negative binomial models for effects on unweighted degree, their ΔAICc values, model weights, and conditional trigamma pseudo R^2 (Nakagawa et al., 2017). All models included the enclosure and data collection method as random effects. Squared variables are orthogonal 2nd degree polynomials of corresponding continuous values. Models were generated using social variables, environmental abiotic variables, individual variables, and combinations of these three variable sets.

Model	Fixed Effects	AICc	ΔAICc	Conditional R^2
Full: Social	**Adult sex ratio, adult sex ratio^2, estimated juvenile mortality, average estimated soil temperature, age class, sex, body weight**	**1253.2**	**0.0**	**0.64**
Social: Sex ratio + juvenile mortality	Adult sex ratio, adult sex ratio^2, estimated	1259.5	6.3	0.62
Individual	Age class, sex, body weight	1283.7	30.5	0.64
Full: Day length	Average estimated soil temperature, day length, age class, sex, body weight	1286.7	33.5	0.65
Full: Rainfall	Average estimated soil temperature, cumulative rainfall, age class, sex, body weight	1287.5	34.3	0.65
Social: Density	Adult population density, adult population density^2	1295.5	42.4	0.56
Null	1 (intercept)	1303.1	49.9	0.59
Abiotic: Day length	Average estimated soil temperature, day length	1306.4	53.2	0.60
Abiotic: Rainfall	Average estimated soil temperature, cumulative rainfall	1306.4	53.3	0.60

Table 3.2: Fixed effect estimates for the full model on unweighted degree and their 95% confidence intervals. Significant effects, as determined by 95% confidence intervals that do not cross zero, are indicated in bold. Voles had higher unweighted degree if they were adults, male, when estimated juvenile mortality was higher, and when the population exhibited a male-biased sex ratio. The estimates and confidence intervals for polynomial variables (i.e., adult sex ratio^2) cannot be directly interpreted but are included below.

Fixed effect	Estimate	95% CI
Age class: Adult	**0.29**	**0.03, 0.56**
Body weight	-0.38	-1.36, 0.60
Sex: Male	**0.21**	**0.07, 0.36**
Average estimated soil temperature	-0.57	-1.82, 0.68
Estimated	**0.56**	**0.27, 0.84**
Adult sex ratio	**-5.71**	**-7.77, -3.65**
Adult sex ratio^2	-0.77	-2.90, 1.36

Table 3.3: Candidate models for predictor effects on degree weight, their ΔAICc values, and conditional R^2 (Nakagawa et al., 2017). All models included the enclosure and data collection method as random effects. Squared variables are orthogonal 2nd degree polynomials of corresponding continuous values. Models were generated using social variables, environmental abiotic variables, individual variables, and combinations of these three variable sets.

Model	Fixed Effects	AICc	ΔAICc	Conditional R^2
Social: Density	**Adult population density, adult population density^2**	122.4	**0.0**	**0.19**
Social: Sex ratio + juvenile mortality	**Adult sex ratio, adult sex ratio^2, estimated juvenile mortality**	123.7	**1.4**	**0.22**
Full: Density	**Adult population density, adult population density^2, age class, sex, body weight**	123.9	**1.6**	**0.22**
Full: Sex ratio + juvenile mortality	Adult sex ratio, adult sex ratio^2, estimated, age class, sex, body weight	127.7	5.4	0.24
Null	1 (intercept)	131.3	8.9	0.10
Abiotic: Rainfall	Cumulative rainfall, average estimated soil temperature, age class, sex, body weight	133.2	10.8	0.09
Abiotic: Day length	Day length, average estimated soil temperature, age class, sex, body weight	133.2	10.8	0.09
Individual	Age class, sex, body weight	133.4	11.0	0.13

Table 3.4: Fixed effect estimates for the social model of density (top) and the social model of sex ratio and estimated (bottom) on unweighted degree, their standard errors, z-scores, and p-values. Significant effects, as determined by p-values < 0.01, are indicated in bold. The estimate and confidence interval for polynomial variables (adult population density^2 and adult sex ratio^2) cannot be directly interpreted but have significant effects in combination with the linear variables.

Social Model: Density

Fixed effect	Estimate	SE	z	p-value
Adult population density	0.70	0.30	2.37	0.02
Adult population density^2	0.92	0.30	3.06	0.002

Social Model: Sex ratio + juvenile mortality

Fixed effect	Estimate	SE	z	p-value
Adult sex ratio	0.36	0.10	3.87	0.26
Adult sex ratio^2	1.24	0.34	2.59	<0.001
Estimated juvenile mortality	0.08	0.05	1.79	0.08

Figure 3.1: Diagram of the two radio frequency identification array configurations (i.e., RFID Array A; RFID Array B) and the trapping stakes where we set our traps, depicted as pink squares, purple squares, and black dots, respectively. We set three traps at each trapping stake. RFID arrays are shown larger than scale for image visibility–each RFID antenna is located at the center of a pink or purple square on the diagram.
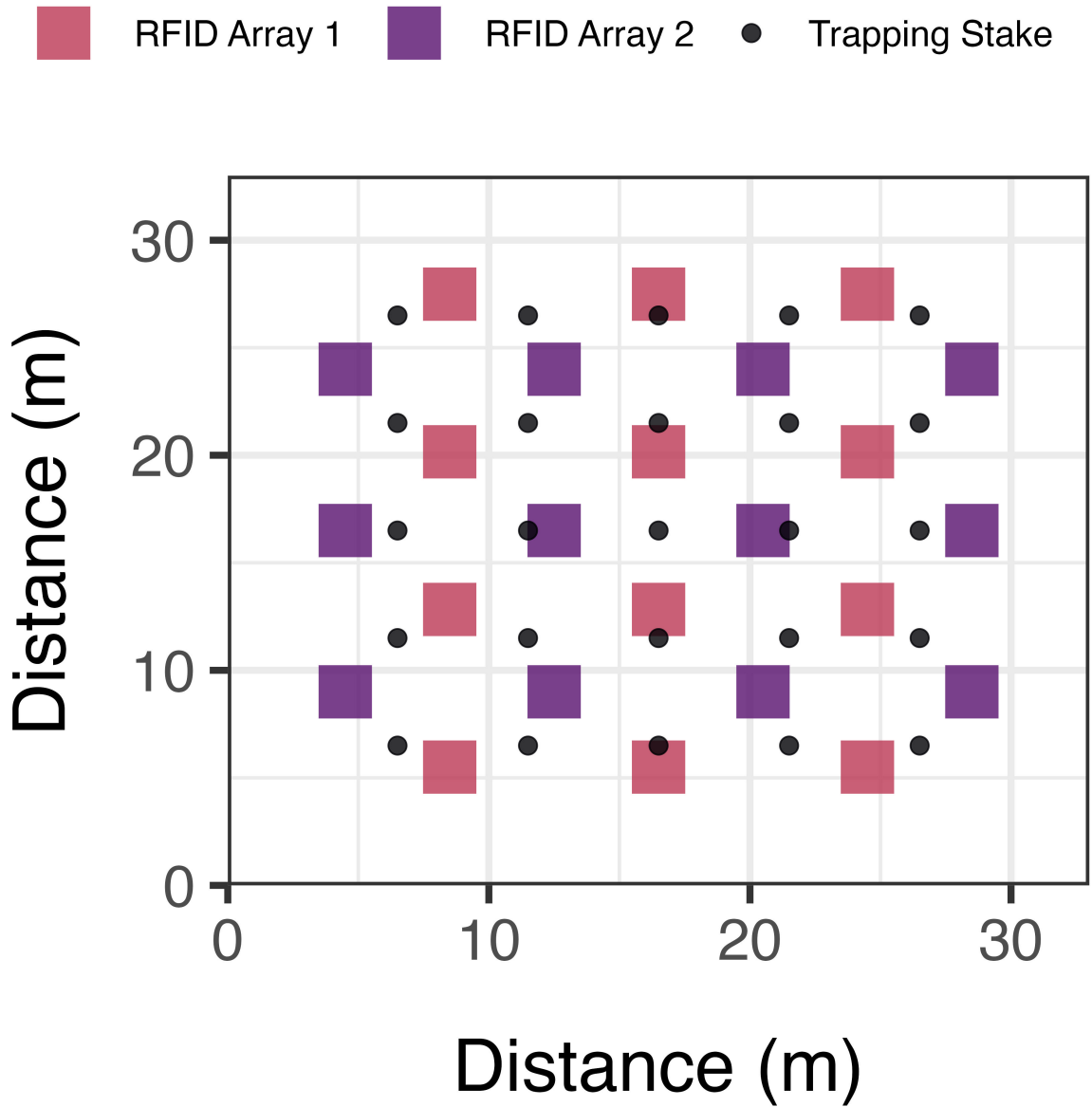
Figure 3.2: A time ordered social network graph showing patterns of association from June 2019 to June 2022, from trapping and RFID data. Purple dots along the x-axis represent individual voles at single time points. Horizontal lines represent weighted edges between individuals at the same time point. Vertical lines represent the same individual at different time points, with increasing time in days progressing along the y-axis. Colored sections of the graph indicate the seasonal time periods that were aggregated for the remainder of the statistical analyses, labeled with cutoff months on the right side of the graph.
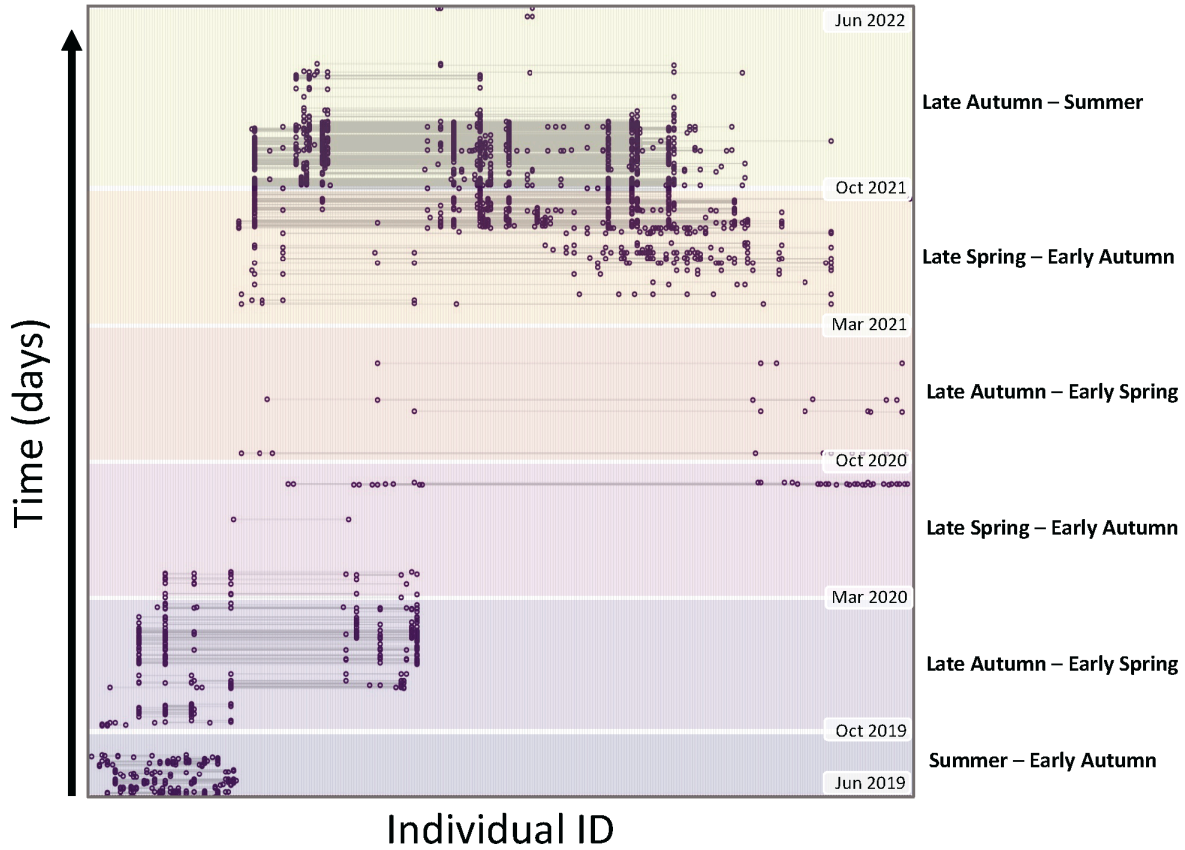
Figure 3.3: Graph visualizations of seasonally aggregated networks, from which summary statistics of unweighted degree and degree weight were generated for further statistical analysis. Colored text boxes above (row 1) and below (row 2) each graph indicate the time period aggregated from Figure 3.2. The boxed number in the center of each graph indicates the number of voles present in each graph (i.e., number of unique vertices). Within each graph, purple circles are nodes, indicating each individual, and blue lines are edges, visualizing network connections. Width of each edge indicates its relative weight to other edges in the same graph, using a simple ratio index (Cairns & Schwager, 1987). Blue arrows between graphs indicate the flow of time. We did not collect trapping data March 2020 - June 2020 due to the COVID-19 pandemic, and we did not collect RFID data April 2020 - July 2020 because the RFID reader was not functional. Populations were established by introducing 48 adult prairie voles to the enclosures in early June 2019, and an additional 28 adult prairie voles were added in late November 2019, as described in *3.3.2 Study Animals*.
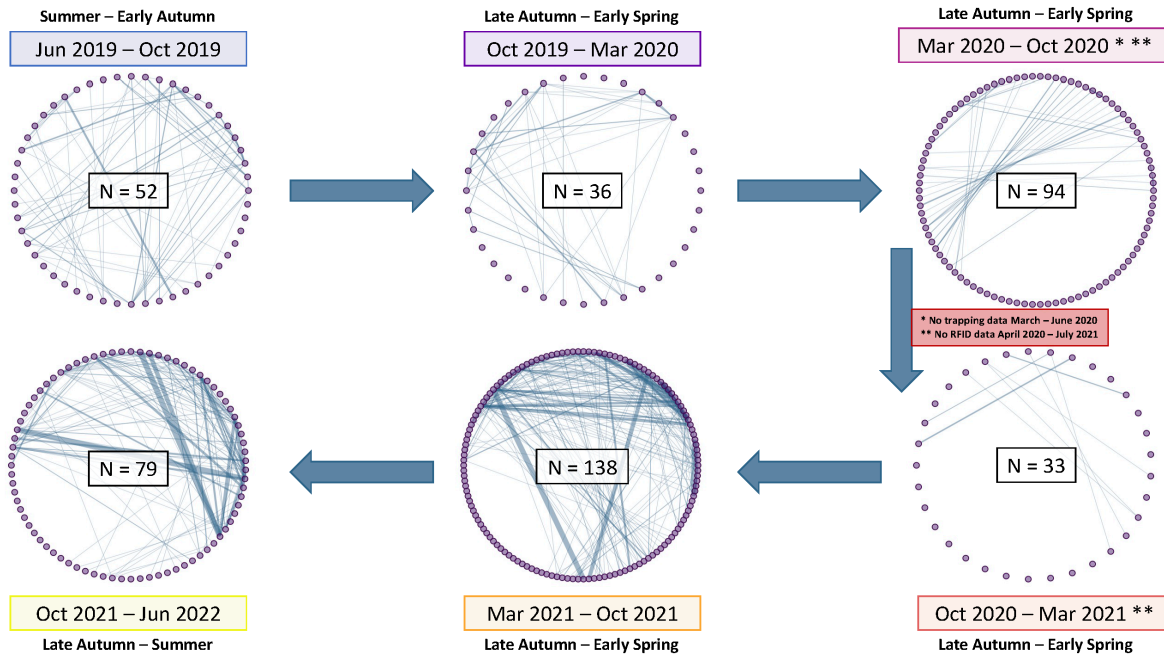
Figure 3.4: A forest plot showing the size of the coefficient for each of the main effects in the full, social model for unweighted degree, and the 95% confidence interval for each estimate. Points falling to the right of the line indicate a positive effect of that predictor on unweighted degree, while points falling to the left indicate a negative effect of that predictor on unweighted degree. A 95% confidence interval, depicted as the capped line around the point, that does not overlap the red dashed line at zero indicates a significant, reliable effect on unweighted degree. Adult age class relates to subadult age class and male sex relates to female sex.

Effect Size on Unweighted Degree
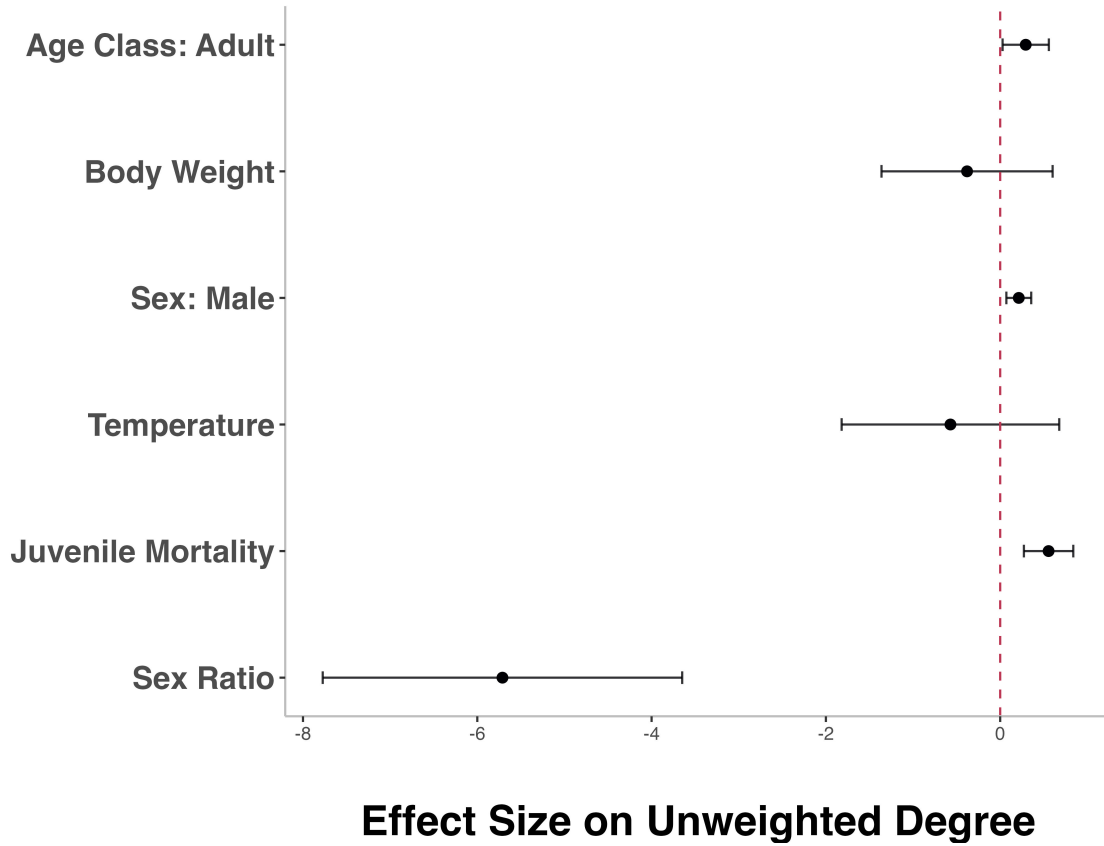
Figure 3.5: Raw data for unweighted degree by age class (left to right: subadult, adult) are shown as jittered points. The lines (subadult in light purple, adult in dark purple) visualize the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018). Adults are predicted to have slightly, but significantly, higher unweighted degree than subadults.
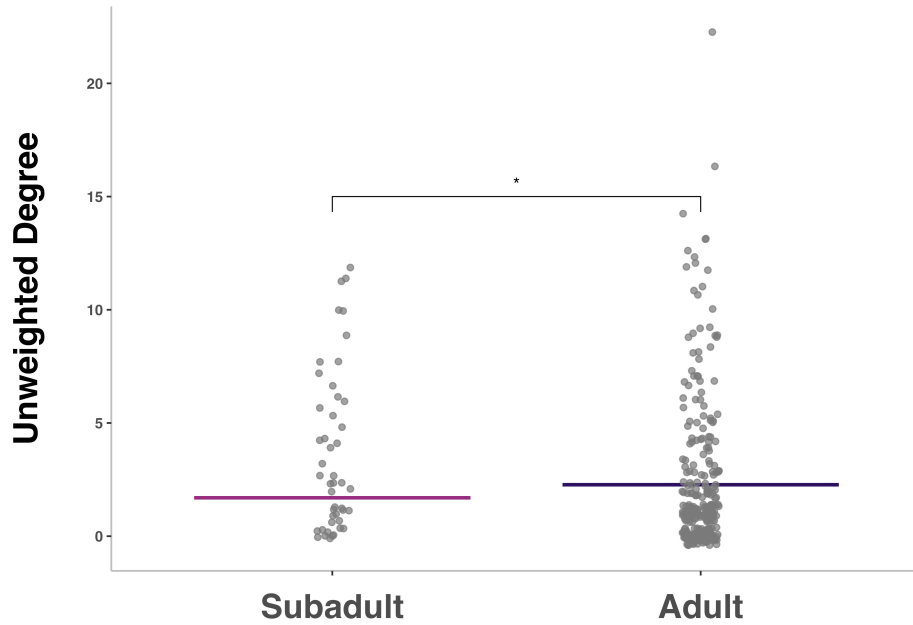
Figure 3.6: Raw data for unweighted degree by sex (left to right: female, adult) are shown as jittered points. The lines (female in light orange, male in dark orange) visualize the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018). Adults are predicted to have slightly, but significantly, higher unweighted degree than subadults.
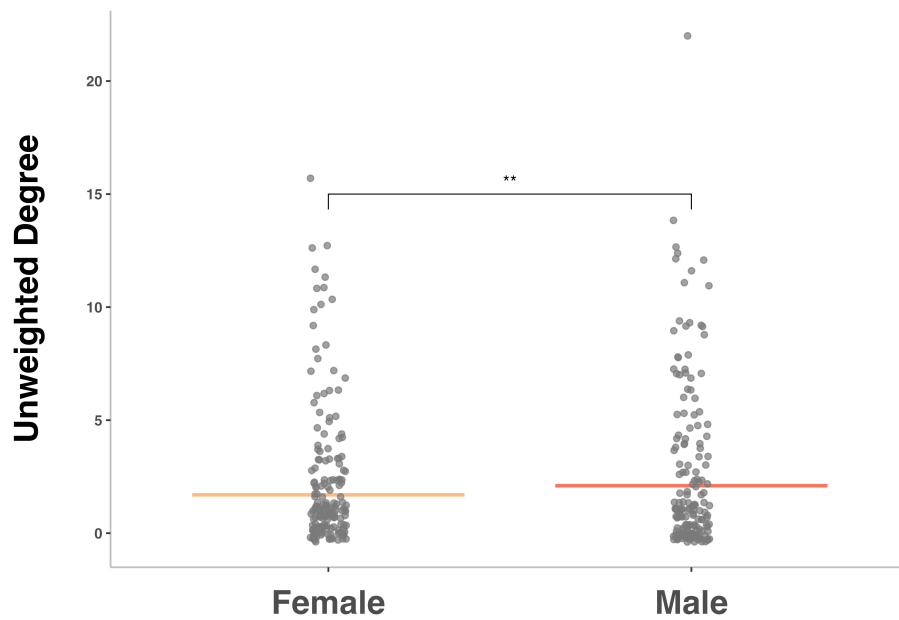
Figure 3.7: Raw data for unweighted degree by estimated juvenile mortality are shown as jittered points. The line visualizes the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018), with the 95% confidence interval of the means. Unweighted degree is predicted to be higher when juvenile mortality is higher.
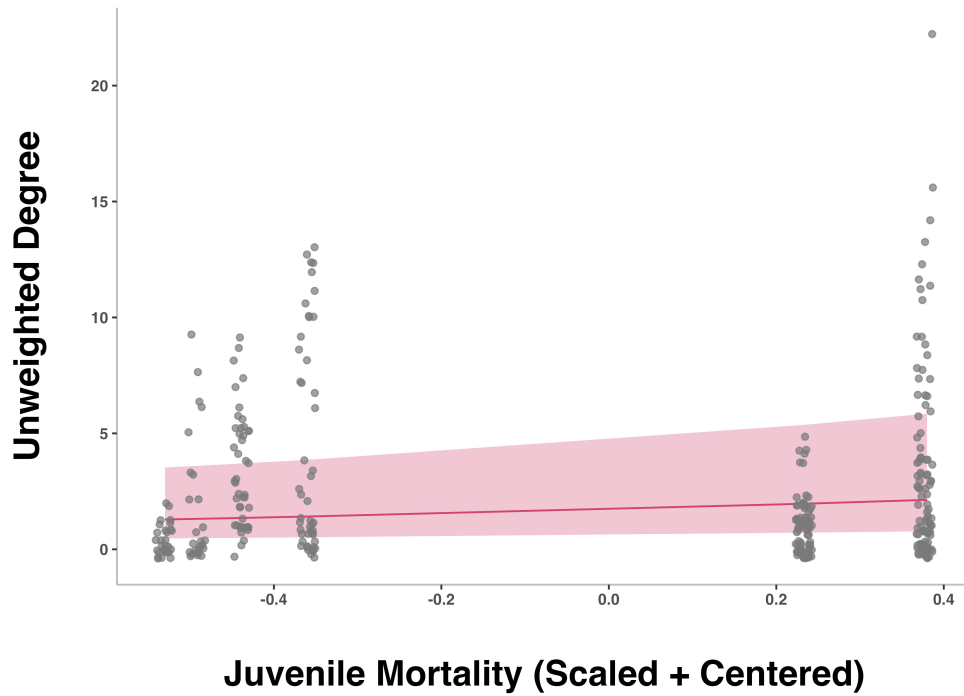
Figure 3.8: Raw data for unweighted degree by population sex ratio are shown as jittered points. The line visualizes the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018), with the 95% confidence interval of the means. Unweighted degree is predicted to be higher when population sex ratio is low (i.e., there are fewer males in the population).
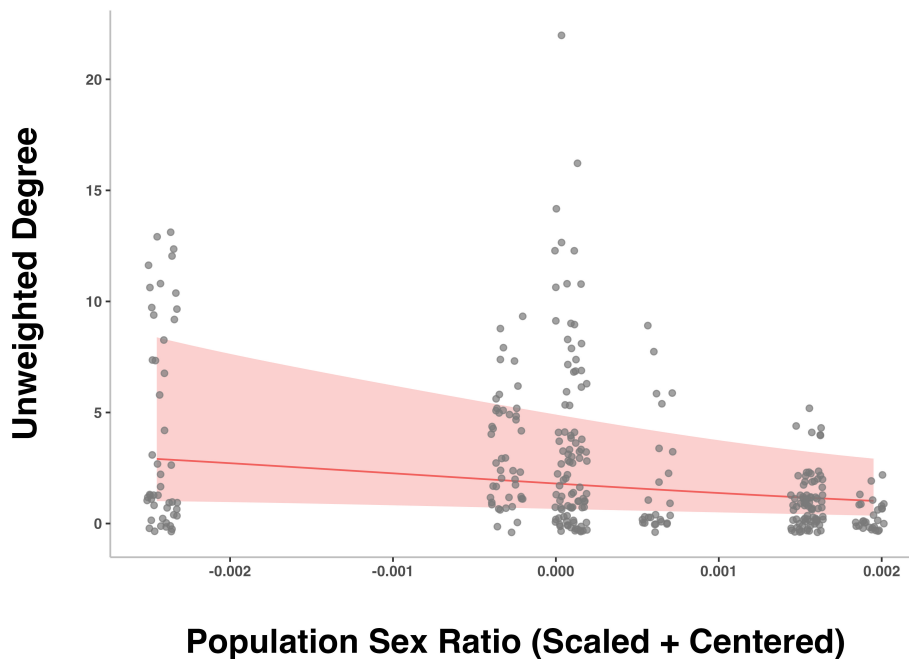
Figure 3.9: Raw data for degree weight by adult population density are shown as jittered points. The line visualizes the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018), with the 95% confidence interval of the means. Degree weight is predicted to be higher when adult population density is at the low or high extremes.
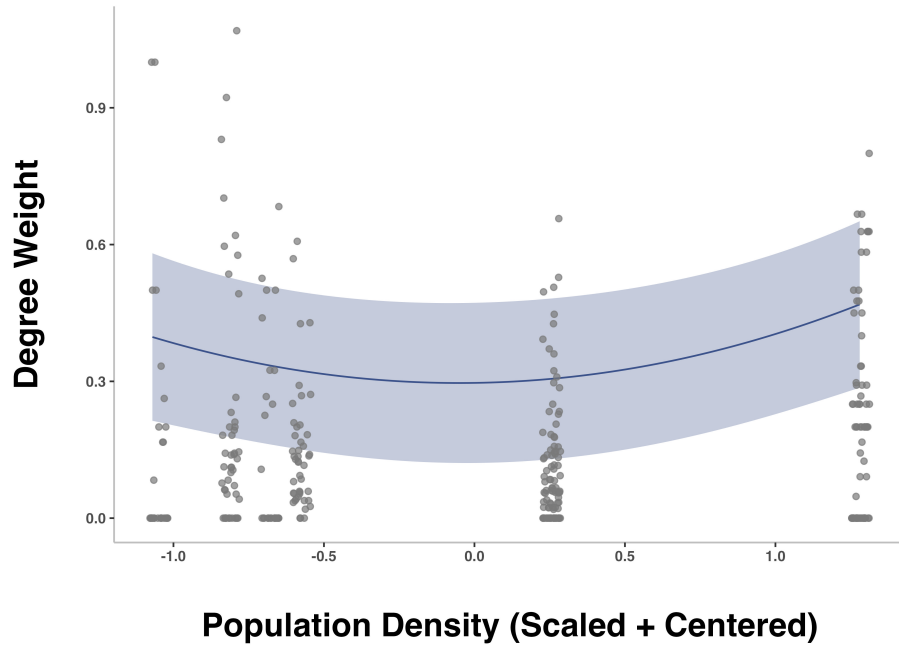
Figure 3.10: Raw data for degree weight by adult sex ratio are shown as jittered points. The line visualizes the means of data predicted using the model by the R package ggeffects version 1.32 (Lüdecke, 2018), with the 95% confidence interval of the means. Degree weight is predicted to be higher when adult sex ratio is at either extreme (i.e., an unbalanced number of males and females).
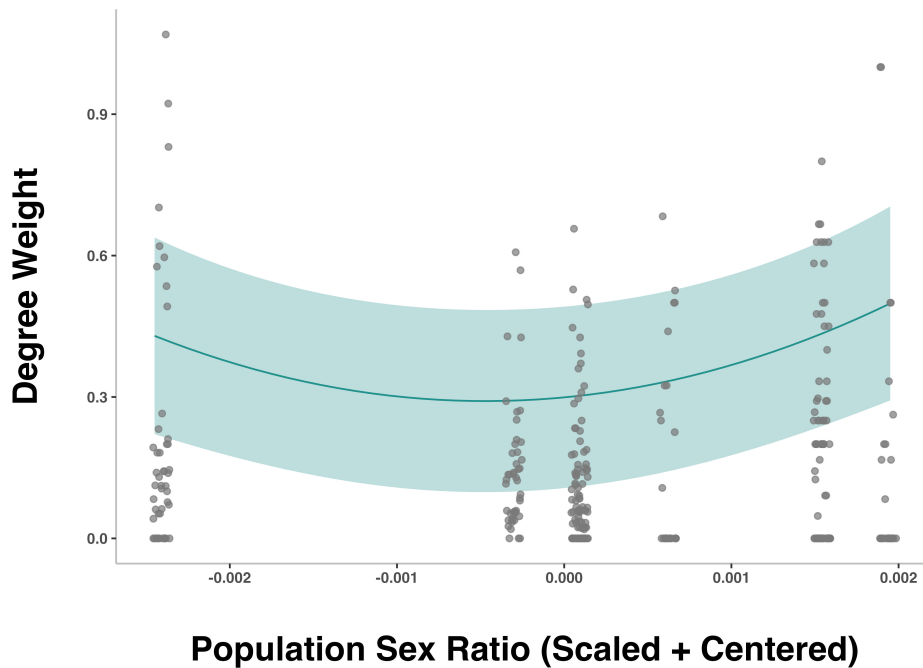
Figure 3.11: Scatter plot of degree weight by adult sex ratio (scaled and centered), split by opposite-sex edges (shown in blue) and same-sex edges (shown in green). We did not detect any significant differences in the relationships between same sex degree weight and opposite sex degree weight with adult sex ratio ($p > 0.05$).
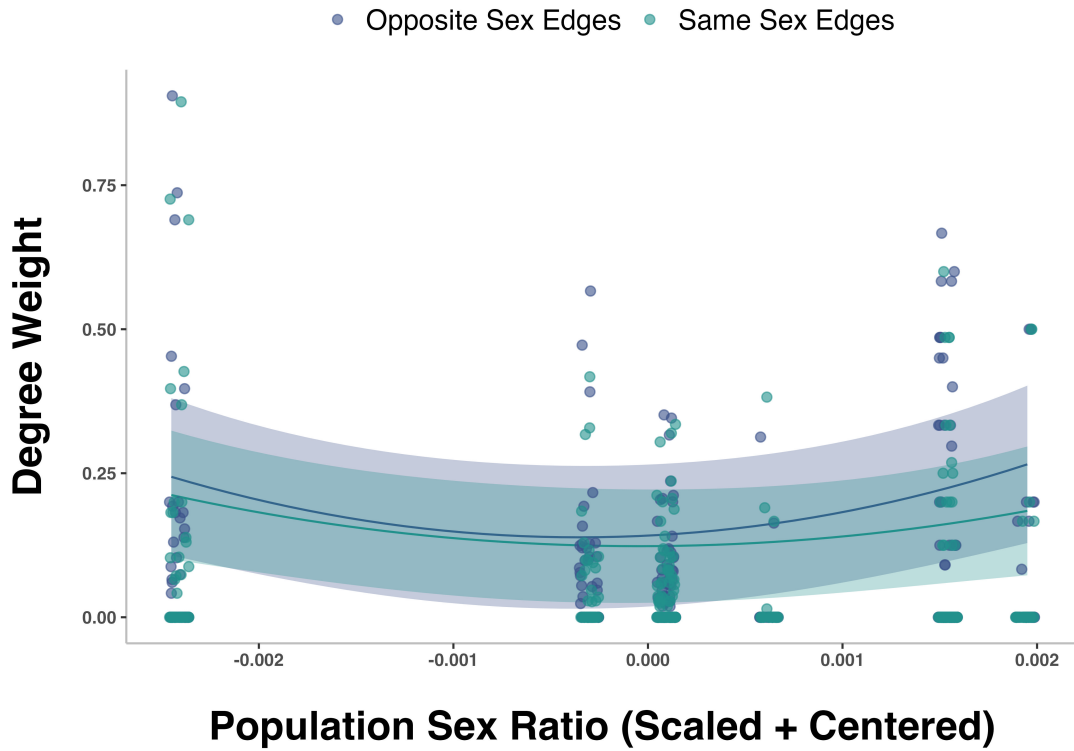
3.9 Supplementary Materials

Supplementary Table 3.1: Details of radio-frequency identification (RFID) array rotation between two configurations (i.e., "A" or "B") and four identical outdoor, semi-natural enclosures (1 – 4). See Figure 3.1 for a visualization of the two arrays relative to the enclosure area. NA indicates that the RFID array was not set up in any enclosure. From 04/23/20 to 07/30/21, the RFID reader was not working, so it was removed from the enclosure for troubleshooting.

Date	RFID Array Moved from:	RFID Array Moved to:
07/01/19	NA	enclosure 1 A
07/04/19	enclosure 1 A	enclosure 1 B
07/06/19	enclosure 1 B	enclosure 2 A
07/08/19	enclosure 2 A	enclosure 2 B
07/10/19	enclosure 2 B	enclosure 3 A
07/12/19	enclosure 3 A	enclosure 3 B
07/14/19	enclosure 3 B	enclosure 4 A
07/16/19	enclosure 4 A	enclosure 4 B
07/18/19	enclosure 4 B	enclosure 4 A
07/20/19	enclosure 4 A	enclosure 4 B
07/22/19	enclosure 4 B	enclosure 2 A
07/24/19	enclosure 2 A	enclosure 2 B
07/26/19	enclosure 2 B	enclosure 2 A
07/28/19	enclosure 2 A	enclosure 2 B
07/30/19	enclosure 2 B	enclosure 4 A
08/01/19	enclosure 4 A	enclosure 4 B
08/03/19	enclosure 4 B	enclosure 2 A
08/05/19	enclosure 2 A	enclosure 2 B
08/07/19	enclosure 2 B	enclosure 2 A
08/09/19	enclosure 2 A	enclosure 2 B
08/11/19	enclosure 2 B	enclosure 1 A
08/13/19	enclosure 1 A	enclosure 1 B
08/15/19	enclosure 1 B	enclosure 1 A
08/17/19	enclosure 1 A	enclosure 1 B
08/19/19	enclosure 1 B	enclosure 2 A
08/21/19	enclosure 2 A	enclosure 2 B
08/23/19	enclosure 2 B	enclosure 1 A
08/25/19	enclosure 1 A	enclosure 1 B
10/05/19	enclosure 1 B	enclosure 3 A
04/23/20	enclosure 3 A	NA
07/30/21	NA	enclosure 3 A
03/06/22	enclosure 3 A	enclosure 1 A
06/05/22	enclosure 1 A	NA

Chapter 4 A Behavioral Comparison of Three Laboratory-Bred Populations of Prairie Voles in Semi-Natural Field Enclosures

4.1 Abstract

Many studies relevant to health, neuroscience, and behavior take advantage of laboratory animals to provide controlled study conditions and reveal causal mechanisms behind the phenomena they investigate. However, studies have found robust behavioral differences among and within genetic lines of laboratory animals, despite efforts to limit genetic diversity and standardize housing conditions of these animals. Even with knowledge of behavioral divergence among laboratory bred populations of animals, there have been few studies investigating the effects of laboratory breeding on wild-caught animals used in behavior experiments. Here, we compare physical traits and several aspects of social behavior among three distinct laboratory-bred sets of voles released into semi-natural enclosures. We found little evidence for behavioral differences among our populations of laboratory-bred voles following their introduction into field settings, despite observed differences in body weight and age of founding voles. Instead, our results support previous findings that environmental factors lead to behavioral divergences among populations of laboratory-bred animals.

4.2 Introduction

Animal research is essential to understanding epidemiological models, disorder symptomatology, and genetic disorders, as well as the evolution and ontogeny of behavior (Bale et al., 2019; Bolker, 2019; Cauchoix & Chaine, 2016; Duckworth, 2008; Yi & Li, 2012).

Laboratory studies aim to minimize confounding effects of both genetics and the housing environment on research outcomes by using highly developed animal models, such as mice (*Mus musculus*) and rats (*Rattus norvegicus*), and standardizing housing protocols (reviewed in Olsson et al., 2003). However, research has suggested that, despite efforts to minimize variation among laboratory animal subjects, experimental results may be biased by the selection of isogenic (inbred) animal strain for each study (Olsson et al., 2003), or even the breeding colony of selected outbred animal stocks (Fitzpatrick et al., 2013; Khoo et al., 2022; Park et al., 2021; Takahashi et al., 2021; Yoon et al., 2018). Genetic differences between groups of animals, even when bred as the same type of animal model (e.g., BALB/c mice; Long-Evans rats), may result in behavioral differences, especially in the presence of small environmental differences that may occur across laboratory environments (Loos et al., 2014; Wahlsten et al., 2003). Researchers using automated home cages for laboratory animals to minimize the individual effects of researcher interaction and handling (e.g., Clemens et al., 2014; Krakow et al., 2010; Loos et al., 2014) have found that confounding effects on behavior cannot be sufficiently minimized by genetic or environmental standardization.

Neuroscience, biomedical, and behavior researchers interested in generalization of their experimental results often use outbred stocks of laboratory animals, because the genetic diversity in these populations resembles that of human populations (Festing, 2010). However, the process of outbreeding animals in multiple separate colonies (i.e., by different vendors) may result in genetic differences between colonies, due to genetic drift or accidental selective breeding (reviewed in Festing, 2010). Studies have repeatedly found evidence of within-stock physiological, neurobiological, and behavioral divergences between cohorts of animals obtained from different vendors (reviewed in Fitzpatrick et al., 2013). One well-known example of within-

stock differences in behavior is the incidence of so-called sign- and goal-trackers in Sprague-Dawley (Fitzpatrick et al., 2013) and Long-Evans (Khoo et al., 2022) rats obtained from different breeding colonies. When training Pavlovian conditioned approach response (i.e., pairing a cue stimulus with a reward), researchers have characterized two distinct behavioral phenotypes: sign-trackers (Hearst & Jenkins, 1974), which attend to the *cue* being presented and goal-trackers (Boakes, 1977), which attend to the *reward* associated with the cue. In the selection of outbred rats for studies of incentive salience or predictive cues (e.g., addiction research), the vendor (e.g. Harlan vs. Charles River; Fitzpatrick et al., 2013), or even the colony within the same vendor (e.g., Charles River K72 vs R06; Khoo et al., 2022), may skew study results.

While the importance of understanding genetic and behavioral differences within laboratory animal strains and stocks prior to experimental inclusion has been emphasized in laboratory research, comparison of these topics in field research has remained underexplored. There is an abundance of research demonstrating geographical differences in behavior among populations of wild animals, notably, work on anti-predator behavior in freshwater fish (e.g., Bell & Foster, 1994; reviewed in Foster & Endler, 1999). Previous studies have also compared differences among wild populations of prairie voles (*Microtus ochrogaster*) in Kansas, Indiana, Illinois, and Tennessee, finding significant differences in female social and reproductive behavior (Solomon & Keane, 2018; Wolff and Dunlap 2002; Wolff et al., 2002), as well as differences in sexual dimorphism and paternal genetic influence on parental care (Roberts et al., 1998), among the different populations. Importantly, behavior is often flexible, resulting in within-population phenotypic spectrums and complicating the study of geographical differences in behavior (reviewed in Foster & Endler, 1999; Lott, 1984). In other words, researchers must determine whether observed differences are due to immediate behavioral adaptation to differing

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conditions or underlying genetic divergences from (often, rapid) natural selection (in Foster &

Endler, 1999; Lott, 1984).

To these ends, a more recent study compared morphology, genetics, and behavior of F1–

F3 generation laboratory-bred prairie voles descended from wild voles trapped in Illinois and

Tennessee and introduced into semi-natural outdoor enclosures at the University of Memphis,

Tennessee (Ophir et al., 2007). However, contrary to previous studies that found behavioral

differences among populations of prairie voles from various regions across the country (Roberts

et al., 1998; Solomon & Keane, 2018; Wolff and Dunlap, 2002; Wolff et al., 2002), this study

found no evidence for differences in affiliation, pair-bonding, home range, or paternal care in

male voles from Illinois and Tennessee, despite genetic differences (Ophir et al., 2007). To our

knowledge, this is the only existing study that explicitly compared the behavior of populations of

laboratory-bred prairie voles with different genetic backgrounds (but the same early-life

conditions) when introduced to semi-natural enclosures. In addition, no studies have compared

behavioral differences among prairie voles bred and maintained at different laboratories under

separate protocols upon introduction to semi-natural field enclosures.

Here, we compare data from prairie voles bred in three different laboratory colonies that

were introduced to semi-natural enclosures to determine whether these groups of voles

demonstrate differences in behavior. Prairie voles are a suitable model for studying the question

of behavioral divergences across stocks of laboratory animals introduced to field settings because

they can be captured regularly from the wild to start laboratory colonies and have been

consistently studied in the wild, the laboratory, and semi-natural enclosures for several decades.

Studies in which laboratory-bred animals are released into outdoor enclosures are common with

this model species, but despite their long-term use in this type of research, little is known about the effects of laboratory breeding on wild-caught voles (but see Ophir et al., 2007).

We hypothesized that there would be behavioral differences among vole populations founded by different laboratory colony stocks, due to the widespread behavioral differences observed among more common rodent models in laboratory settings. However, due to the inconsistency of results demonstrating differences among laboratory stocks of mammals in field studies, we focused on comparing physical traits of the individuals from each breeding colony we obtained animals from and conducting exploratory analyses of social behavior, including home range area, mating tactic (i.e., behavioral phenotype), and social network measures of the number and strength of social contacts.

An animal's home range area encompasses the locations that they regularly attend to, including their nest, home territory, and preferred foraging locations (Powell & Mitchell, 2012). This measure provides an estimate for researchers to understand how individuals relate to the surrounding resources and construct cognitive maps of their environment (Powell & Mitchell, 2012; Rice et al., 2022). We measured home range area and mating tactic, assigned by home range area overlap, to replicate analyses from a previous study that compared space use and mating behavior across populations of genetically different laboratory populations of prairie voles (Ophir et al., 2007). In addition to these measures, we were also interested in social network metrics of behavior that provide information about the number and strength of social contacts among individuals in a population. Social network analyses (SNA) allow researchers to understand social structures at the individual and population levels, as well as identify behavioral strategies and dynamics (Krause et al., 2007). With this combination of methods, we aimed to

characterize potential behavioral differences across prairie vole populations with multiple perspectives.

4.3 Methods

4.3.1 Study Area

We conducted all fieldwork for the study at the Miami University Ecology Research Center (ERC) in Oxford, Ohio (39° 53'N, 84° 73'W). Fieldwork for this study took place over three summer seasons (8 months total; May–Aug 2017, June – Aug 2019, and May – Aug 2023).

We established populations in the same set of enclosures for all of these fieldwork periods, which consisted of two to four 0.1 ha (32m x 32m) semi-natural outdoor enclosures at any time. The enclosures were surrounded by 20-gauge galvanized steel walls, which extended 75 cm aboveground and 45 cm belowground to prevent voles from digging out of the enclosure (Cochran and Solomon, 2000). An electrified wire ran along the top of the enclosure walls to exclude terrestrial predators, such as raccoons (*Procyon lotor*) and domestic house cats (*Felis catus*), from the study area and discourage birds from perching around the edges. However, the enclosures were open air and uncovered from the top, so birds of prey could hunt from above and large snakes could probably enter the enclosures over the top of the walls. We regularly checked the condition of the walls for holes and mowed an approximately 1-m wide strip at the inner edge of each enclosure to discourage digging.

Vegetation in all enclosures consisted of goldenrod (*Solidago* spp.), clover (*Trifolium* spp.), timothy (*Phleum* spp.), ryegrass (*Elymus* spp.), fescue (*Festuca* spp.), bluegrass (*Poa pratensis*), and ragweed (*Ambrosia* spp.), which provided natural food and cover for voles (Solomon et al., 2009; **Supplementary Table 2.1**). Prior to the release of any research animals, all enclosures were verified to be empty by live trapping until each had been trapped for three

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consecutive days without catching any animals (e.g., voles, mice, shrews, etc.). We occasionally caught deer mice (*Peromyscus* spp.; N = 16) or short-tailed shrews (*Blarina brevicauda*; *N* = 4) during our data collection period, and they were immediately released outside of the enclosure, without handling.

4.3.2 Study Animals

We used three different laboratory colonies of prairie voles to found each of our sets of populations in the enclosures (i.e., May 2017; June 2019; May 2023). We also obtained voles from a fourth colony (Alex Ophir Laboratory, Cornell University, Ithaca, NY) in November 2019, but we did not include these data here because they were introduced to the enclosures under conditions that are not directly comparable (i.e., during late autumn, into established populations). We never housed, transported, or released opposite-sex siblings into the same enclosure, to avoid inbreeding. All founder voles were marked with passive integrated transponder (PIT) tags (Biomark: Boise, Idaho; 12mm HPT tags) implanted subcutaneously behind the shoulders prior to release, which enabled us to uniquely identify all voles for the course of the study and/or their lifetimes. Animals received only a small amount of cracked corn from baiting traps, which provides very little nutrition and caloric value to their diet (Desy & Batzli, 1989). All populations were maintained continuously in the outdoor enclosures for the course of each study, until they were removed and humanely euthanized (in August 2017, June 2022, and August 2023). Details of each founding group of voles are provided below.

4.3.2.1 May – August 2017: Solomon Voles

In late May 2017, we introduced 72 adult, laboratory-bred prairie voles from the Nancy Solomon–Brian Keane Laboratory prairie vole colony at Miami University (Oxford, OH). These voles were part of the F7–F8 generation of animals originally captured in Illinois. Prairie voles in

this colony were kept at a temperature of 19 $\pm$ 1 °C and humidity of 55%, with a 14:10 h

light:dark cycle. Offspring were housed with their parents in 23 × 44 × 21 cm polycarbonate

cages until 21–22 days of age, when they were weaned and housed in pairs (in 17 × 28 × 13 cm

polycarbonate cages) or groups of more than two littermates (in 23 × 44 × 21 cm polycarbonate

cages). All voles were housed with processed paper bedding (Cell Sorb Plus, A & W Products,

Inc., New Philadelphia, OH, or Softzorb, Northeastern Products Corp. Warrensburg, NY), a

cotton Nestlet (Ancare Corp., North Bellmore, NY), and either dried alfalfa or straw with a

compressed alfalfa block. Food (Rodent Breeder Diet 5013, supplemented once per week with

Hi-Fiber Rabbit Diet 5326, PMI Nutrition International, Brentwood, MO) and tap water were

provided ad libitum. All housing procedures were approved by the Miami University

Institutional Animal Care and Use Committee.

We released 48 voles (24 males and 24 females) into one semi-natural enclosure and 28

voles (14 males and 14 females) into a second semi-natural enclosure. Voles were introduced at

two densities due to a simultaneous, unrelated study not detailed here; however, both enclosures

were started within the density range found in wild populations of prairie voles, based on

previous research (Getz et al. 1993, 2001). Two females and one male were replaced in the first

week of the study with same-sex voles from the same laboratory colony, after being confirmed

dead within the enclosures. Finally, for the duration of this study (May–August 2017), offspring

remained in the enclosures but were not included in analyses.

4.3.2.2 June – August 2019: Grippo Voles

In early June 2019, we released 48 adult, laboratory-bred prairie voles, part of the F2–F3

descended from voles originally captured in Illinois and bred by the Angela Grippo Laboratory at

Northern Illinois University (DeKalb, IL), into four identical, semi-natural enclosures. Prairie

voles in this colony were kept at a temperature of 21–22 °C and humidity of 40–50%, with a 14:10 h light:dark cycle. Offspring were housed with their parents in 25 × 40 × 60 cm tinted polysulfone cages with wire tops (Ancare Corp, Bellmore, NY) until 21 days of age, when they were weaned and housed in same-sex pairs in 12 × 18 × 28 cm tinted polysulfone cages with wire tops (Ancare Corp, Bellmore, NY). All voles were housed with aspen chip bedding (Northeastern Products Corp. Warrensburg, NY), and a square of cotton nesting material (Ancare Corp., North Bellmore, NY). Food (Purina rabbit chow, Purina, St. Louis, MO) and filtered water were provided ad libitum. All housing procedures were approved by the Northern Illinois University Institutional Animal Care and Use Committee and conformed to the National Institutes of Health guidelines in the Guide for the Care and Use of Laboratory Animals.

We distributed voles such that each of the four enclosures had founding populations of six males and six females, equivalent to a medium density natural population of 120 voles/ha, based upon density measurements by Getz et al. (1993, 2001). We did not replace any voles who died during the duration of the study (June–August 2019), and voles were allowed to breed naturally. Offspring were PIT tagged when they reached a weight large enough to safely tag (body mass of ~12 g, measured using a spring scale; MD personal observation). Voles were moved between populations in July and August 2019, to reduce possible inbreeding and attempt to equalize sex ratios across the enclosures. To do this, we censused all populations by intensively trapping the enclosures over two weeks. We then selected subadults (individuals weighing between a juvenile weight of 20 g and an adult weight of 30 g) of the correct sex (using trapping records of visually confirmed sex) and relocated them to the center of another enclosure for release immediately after being caught in a trap.

4.3.2.3 May – August 2023: Burkett Voles

In early May 2023, we released 24 adult voles (12 males, 12 females) of the F3 generation, descended from voles originally captured in Illinois and bred in the James Burkett Laboratory at the University of Toledo (Toledo, OH) into two semi-natural enclosures at the ERC. Prairie voles in this colony were kept at a temperature of 20.5–22.7 °C and humidity of 30–50%, with a on a 12:12 h light:dark cycle. Offspring were weaned at 19–21 days of age and housed in groups of 2–3 same-sex voles in 29 × 40 × 19 cm ventilated rat cages (Allentown, Allentown, NJ). All voles were housed with paper bedding (Pure o'Cel, The Andersons, Maumee, OH), and provided a Bed r'Nest (The Andersons, Maumee, OH), Nestlet (Ancare Corp., North Bellmore, NY), CrawlBalls (Bio-Serv, Flemington, NJ), and 500g Apple Sticks (Amazon [Bojafa], Seattle, WA) for nesting and chewing enrichment. Food (Rabbit Chow 5326, LabDiet, St. Louis, MO) and tap water were provided ad libitum. All housing procedures were approved by the University of Toledo Institutional Animal Care and Use Committee and the National Research Council's Guide for the Care and Use of Laboratory Animals.

We did not replace or add any additional voles after the start of the study (in May 2023). Voles were allowed to freely breed, but we removed juvenile offspring from the enclosures and humanely euthanized them to avoid increases in vole density over the course of the season.

4.3.3 Live Trapping

We used live trapping (hereafter, "trapping) with each set of animals in this study to collect social interaction data, track body condition, and capture offspring (either for PIT tagging or humane euthanasia; see *4.3.2 Study Animals* for full details). We placed traps on a 5 m x 5 m grid in each enclosure, with three multiple-capture Ugglan traps (Granhab: Gnosjö, Sweden) at each grid location (i.e., 75 traps per session; see **Figure 2.1**). When we handled each captured

vole, we scanned its PIT tag with a handheld transponder to record its unique identity, recorded

capture location as the trapping stake on the 5 x 5 grid (**Figure 2.1**), sex by visual confirmation,

and body weight (g) to the nearest 0.5 g using a spring scale (Pesola: Schindellegi, Switzerland).

We also recorded the PIT tag IDs of each other animal captured in the same trap and/or at the

same trapping stake, using a handheld transponder.

We trapped each enclosure one to three times per week, either setting traps in the early

evening (1700–2000 h) and checking them 2–4 hours later or setting traps in the late evening

(2000–2300 h) and checking them 8–10 hours later. We did not trap during heavy rain or

thunderstorms and rescheduled missed trapping sessions later in the week when possible.

4.3.4 *Radio Frequency Identification (RFID) Data*

During May–August 2017 and June–August 2019 we used a passive RFID monitoring

system to record movement of PIT tagged voles throughout the enclosures. This system

consisted of a central RFID Reader (Biomark: Boise, Idaho; RM310/SM303 System) connected

to 12 antennas dispersed throughout the enclosure on one of two 3 x 4 arrays, with antennas

spaced either 8 or 7.33 m from each other (**Figure 3.1**), which detected PIT tags of any vole

within 1–3 cm of the antenna surface (MD, personal observation) once per second (Sabol et al.,

2018). We inferred social contact data through patterns of spatial-temporal overlap, measured by

the lag time between two consecutive detections of different PIT tags at the same antenna

(details in **4.3.5 Construction of Social Networks**)

We only had one RFID system, so it was rotated among enclosures throughout the study

periods. The antennas were also rotated through two different array configurations to measure

activity throughout the maximum area in the enclosures, as described in Sabol et al. (2018) and

shown in **Figure 3.1**. From May to August 2017, the RFID system was rotated every three days through the two antenna array configurations and between enclosures every six days. From June to August 2019, the RFID system was rotated through the two antenna array configurations every two days and among enclosures every 4–8 days. See full details of the RFID rotation in **Supplementary Table 3.1**.

4.3.5 Construction of Social Networks

We constructed two sets of social networks for the comparison of two aspects of social behavior among enclosures: 1) the number of unique social contacts assigned to each vole, known as unweighted degree, and 2) the strength of those social contacts, known as degree weight. Degree weight was calculated using the simple ratio index (SRI), which is the ratio of the number of times that a pair was recorded together to the total number of times each individual was recorded (Cairns & Schwager, 1987). For the first set of networks, we used the live trapping data from 2017, 2019, and 2023 to assign social contacts (i.e., social network edges) to dyads of individuals that were caught in the same trap or at the same trapping location during a single trapping session, indicating spatial-temporal overlap.

We used the RFID data collected in 2017 and 2019 to construct the second set of social networks. To do this, we ran the continuous temporal stream of individual RFID detections through a Gaussian mixture model (GMM), which used antenna location and time in seconds from the start of the recording period to create grouping events based on spatial and temporal overlap (Psorakis et al., 2012). The GMM created a matrix of K groups x N individuals, with all voles sorted by the model into the same group assigned pairwise social contacts, weighted by the SRI (Cairns & Schwager, 1987; Farine, 2013). The GMM assigned animals into groups with lag times of 0–81,551 seconds. Despite the high variability in the range of lag times between

detections considered to be social contacts by our GMM, but we did not threshold the data (i.e.,

assign a cutoff for assigned contacts to be considered valid, such as within 10 min). The benefit

of using a GMM for detecting likelihood social contacts is that researchers do not have to set an

arbitrary time threshold or cutoff—the algorithm determines this cutoff through permutations of

the raw data stream (Psorakis et al., 2012, 2015). See **Figure 2.5** for the full distribution of these

data. Therefore, if two voles were detected within this time range at the same RFID antenna, they

would be assigned a social contact, weighted by the number of observations of that social pair,

the number of total observations of each pair member, and the number of times that both

individuals had been observed at the same time but not together (Hoppitt and Farine, 2018).

We summarized and compared the number of unique connections per individual

(unweighted degree) and the strength of those connections (degree weight) for each individual

vole observed by live trapping from May–August 2017, June–August 2019, and May–August

2023. We also compared unweighted degree and degree weight for each individual vole detected

by the RFID system from May–August 2017 and June–August 2019.

4.3.6 Statistical Analyses

Prior to the construction of our social networks, we compared individual traits of the

founding voles from each laboratory colony (i.e., Solomon, Grippo, and Burkett) prior to

introduction into our field enclosures. We used a linear model to analyze the interaction between

laboratory of origin and age, on weight, to understand patterns in the relation of weight to age

among the three groups of voles. Following introduction of the founding voles to the enclosures,

we calculated the proportion of animals that survived to the end of the study period, which we

analyzed using a chi-square test.

We also compared home range size and overlap of the voles bred in the Nancy Solomon-Brian Keane (hereafter, "Solomon-Keane voles"), Angela Grippo (hereafter, "Grippo voles"), and James Burkett (hereafter, "Burkett voles") laboratories from the live trapping data. We created convex hull polygons for each home range, calculated with the R base package grDevices (version 4.3.1) using the x and y coordinates of the unique locations where each vole was trapped, using the R package splancs (version 2.01-44; Rowlingson & Diggle, 2023) to calculate the area of each convex hull. In short, a convex hull polygon is the smallest polygon that encloses all provided points on the xy coordinate system (Baíllo & Chacón, 2021). Convex hull polygons have been commonly used in behavioral ecology as a simple and common estimate of space use and home range size (Mohr, 1947; Powell, 2000; Row & Blouin-Demers, 2006; White & Garrott, 1990).

Average home range size was calculated for all individuals captured at three or more unique locations and compared among the populations of Solomon-Keane voles, Grippo voles, and Burkett voles using a linear mixed model run in R with the packages lme4)version 1.1-35.1; Bates et al., 2015) and lmerTest (version 3.1-3; Kuznetsova et al., 2017). We also assigned mating tactics as either monogamous residents or promiscuous wanderers by the degree of home range overlap with opposite-sex individuals, following methods detailed by Rice et al. (2022). In short, we used the proportion of home range overlap between every possible dyadic combination of voles to calculate encounter rates. We then selected the highest encounter rate for each individual and divided it by the sum of encounter rates for that same individual with all other opposite-sex individuals in the population. If the calculated proportion was higher than 0.5 for the focal vole and its closest opposite-sex social partner, and vice versa, both individuals were considered residents. In other words, voles were considered monogamous residents when their

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home ranges shared over half the area that their home ranges shared with all other voles. In any other case (e.g., voles did not overlap with others, voles did not share more than half of their overlap with a single vole, etc.), voles were considered wanderers.

We also measured home range overlap between the Solomon-Keane voles and the Grippo voles, using RFID data. In this case, we calculated minimum convex hull polygons from the x and y coordinates of every unique RFID antenna that recorded each individual, as per Sabol et al. (2018). We used the R packages sp (version 2.1-2; Pebesma & Bivand, 2005; Bivand et al., 2013), adehabitatHR (version 0.4.21; Calenge & Fortmann-Roe, 2023), and sf (version 1.0-14; Pebesma, 2018; Pebesma & Bivand, 2023) to create minimum convex hull polygons and measure their area. Then, we used a general linear model run with R packages lme4 (version 1.1-35.1; Bates et al., 2015) and lmerTest (version 3.1-3; Kuznetsova et al., 2017) to compare the average home range areas between the Solomon-Keane voles and the Grippo voles.

Finally, we compared summarized unweighted degree and degree weight between the Solomon-Keane voles, the Grippo voles, and the Burkett voles, using the two social networks we constructed (details in *4.3.5 Construction of Social Networks*) from trapping and RFID social contact data, respectively. We used zero-inflated Poisson regression run in R package glmmTMB (version 1.1.8; Brooks et al., 2017) to compare unweighted degree (zero-inflated count data). With our methods, all individuals recorded alone at an RFID antenna (but never assigned a social contact) had a degree of zero; however, we could not rule out the possibility of missing data that would account for these zeros. We used a linear model run with the R packages lme4 version 1.1-35.1 (Bates et al., 2015) and lmerTest version 3.1-3 (Kuznetsova et al., 2017) to compare weighted degree, adjusted by an arcsine transformation to adjust proportion data to meet

assumptions for use in linear models (Sokal & Rohlf, 1995; Zar, 1998; Gotelli & Ellison 2004; but see Warton & Hui, 2011).

4.4 Results

4.4.1 Individual Traits of Founding Voles

There was a main effect of age on body weight, such that older voles were also heavier ($\beta = 0.23$; $p = 0.003$). There was also a significant interaction between age and laboratory of origin, such that Burkett voles were heavier than Grippo ($\beta = -0.25$; $p = 0.009$) or Solomon-Keane ($\beta = -0.22$; $p = 0.004$) voles for their age (**Figure 4.1**). In other words, Burkett voles were approximately 0.2–0.25 g larger than Grippo or Solomon-Keane voles at the same weight.

4.4.2 Survival Analyses

There were no significant differences in the survival of Burkett, Grippo, or Solomon-Keane voles to the end of the study period (chi-square test; $X^2 = 4.33$; $p = 0.115$; **Figure 4.2**). Within the vole populations, 27% of Burkett voles, 23% of Grippo voles, and 13% of Solomon-Keane voles survived until the end of the study period (**Figure 4.2**).

4.4.3 Home Range Comparisons

There were no significant differences between the home range areas (depicted in **Figure 4.3**) of Burkett voles and Grippo ($p = 0.40$) or Solomon-Keane ($p = 0.20$) voles, when we estimated home range area from the coordinates of live trapping locations (**Figure 4.4**). Home range areas varied from 0.0 m^2 (i.e., individuals were trapped at fewer than three unique locations, so a convex hull polygon could not be created) to 237.5 m^2. There were also no significant differences in the distribution of residents and wanderers among the three populations

(chi-square test; $X^2 = 4.91$; $p = 0.09$; **Figure 4.5**) Within the populations voles, 22% of Burkett voles were, 40% of Grippo voles were residents, and 13% of Solomon-Keane voles were residents (**Figure 4.5**).

We also compared the home range areas of voles bred in the Solomon and Grippo Laboratories, estimated using coordinates of RFID antenna locations. Using this method of estimation for home range area, we found that the voles bred in the Solomon Laboratory had lower average home range areas than the voles bred in the Grippo Laboratory ($p = 0.0008$; **Figure 4.6**). Voles bred in the Solomon Laboratory had home ranges 0.7 cm^2 smaller than voles bred in the Grippo Laboratory—though this difference was significant, it is unlikely to be biologically meaningful.

4.4.4 Comparisons of Unweighted Degree and Degree Weight

Using social network summary values generated from spatial-temporal co-occurrence during live trapping, Solomon-Keane voles had significantly higher average unweighted degree than Grippo or Burkett voles ($p < 0.0001$; **Figure 4.7**). On average, Solomon-Keane voles had 1.6 more unique social contacts than the other groups of voles, even when controlling for enclosure differences (**Figure 4.7**). In the same social networks, Solomon-Keane voles also had 0.18 higher average degree weight than Grippo or Burkett voles ($p < 0.0001$; **Figure 4.8**).

Similarly, when we compared unweighted degree and degree weight summarized from social networks constructed with instances of spatial-temporal co-occurrence recorded via the RFID system, we found that Solomon-Keane voles had an average of 1.9 more social contacts than Grippo voles ($p < 0.0001$; **Figure 4.9**). However, this difference in unweighted degree did

not correspond to a significant difference in degree weight between the two groups of founding voles (p = 0.061; **Figure 4.10**).

4.5 Discussion

Here, we compared several aspects of behavior among laboratory voles bred in different laboratory colonies, to determine whether laboratory breeding affects behavior under semi-natural conditions. Based on previous research, showing differences in laboratory rat stocks from different breeding vendors (Fitzpatrick et al., 2013) and colonies within vendors (Khoo et al., 2022), we expected to find differences in behavior among our founding voles once introduced to the semi-natural enclosures. Although there were qualitative differences in the early life experiences (e.g., bedding type) and quantitative differences in the relationship between age and weight (**Figure 4.1**) of the cohorts of voles that we obtained from different laboratories, they did not result in significant differences in survival to the end of the study period (**Figure 4.2**), home range size as measured by trapping (**Figure 4.4**), or the proportions of individuals assigned resident or wandering mating tactics using those measure of home range overlap (**Figure 4.7**). Together, these findings suggest that differences in early life environment, stemming from variation in laboratory breeding protocols, do not affect the natural space use behaviors that researchers are interested in when conducting semi-natural enclosures, nor do differences in individual traits such as age and body weight. However, when we measured home range area using RFID data, we found that the Grippo voles had higher home ranges than the Solomon voles (**Figure 4.6**).

Home range area is an important measure of space use for animals, which provides an estimate for researchers to understand how individuals relate to and remember the surrounding resources (Powell & Mitchell, 2012; Rice et al., 2022). Importantly, estimates of home range

area calculated by convex hulls—the method we used in this study—may provide an inaccurate estimate of the places where animals spend their time, especially when the sample size of unique spatial coordinates is small (Powell & Mitchell, 2012). Because we calculated home range area with a minimum of three unique points, our home range areas are likely missing essential information about the space use of our study animals. In addition, trapping relies on animals being drawn to baited traps and, while the calculations of home range using trapping locations likely record important information about foraging behavior, they may miss recording random behavior that is not motivated by food. Conversely, RFID antennas record a random sampling of activity as the prairie voles move throughout the enclosure, whether foraging, searching for mates, or otherwise behaving. This difference between the specificity of methods that we used could explain why we recorded a difference in home range size calculated using RFID detections, but not trapping.

When we considered patterns of spatial-temporal overlap among individuals, calculated through SNA, we found that that the Solomon-Keane voles reliably had a higher number of unique social contacts than the Burkett or Grippo voles. When we obtained the voles from the Solomon-Keane laboratory, we were conducting a concurrent, unrelated study that investigated differences in behavior between a high-density and a low-density population of voles. Therefore, these two populations were established at population densities 2–4 times higher than the densities of the populations founded using the Burkett and Grippo voles. Although we included enclosure (i.e., population identity) in our social network models as a random effect, to control for differences in confounding factors such as vegetation, population density, and year, it is plausible that our results merely reflect the effects of high density on social contacts. Because the Solomon-Keane voles were maintained at such high densities, both the overlap among territories

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and the likelihood of encountering neighboring conspecifics should be intrinsically higher. Indeed, the polymorphic behavior hypothesis (Chitty, 1960, 1967) suggests that animals at high densities should have frequent, antagonistic interactions, as part of an internal mechanism to regulate population density. Unfortunately, we are unable to test this hypothesis with our current data set because age, weight, and density were all different across our three populations and we cannot separate the causal effects of these factors without manipulating them.

However, in **Chapter 3** of this dissertation, we found that high population density conditions predicted a high number and strength of individual social contacts. Therefore, we speculate that the divergences that we found among vole populations bred from different laboratory colonies in home range and social connections may be due to differences in population density at enclosure establishment. Rather, we suggest that the selection of colony prairie voles for studies in semi-natural enclosures does not have a large effect on the results of behavioral measures, on its own. Here, we did not find evidence of the same level of behavioral divergences in our field studies as has been reported among studies of laboratory mice and rats, even when outbred. For example, we could expect, based on the differences in distribution of sign- and goal-tracking outbred rats obtained from two colonies (Khoo et al., 2022), that we would find differences in the distribution of mating tactics in our prairie voles bred in different colonies; however, that was not the case. Instead, we found more support for a previous prairie vole study that found no differences in laboratory-bred animals originally caught in Illinois and Tennessee, suggesting that geographical and genetic differences in behavior were drowned out under laboratory breeding and semi-natural conditions (Ophir et al., 2007).

Indeed, studies of laboratory mice and rats have suggested that, although genetic divergences exist, behavioral differences between and within groups of animals are largely due

to environmental effects that we might consider negligible (Krackow et al., 2010; Loos et al., 2015; Olsson et al., 2003; Richter et al., 2011; Wahlsten et al., 2003). Therefore, under semi-natural conditions, the extreme variability in habitat and behavior (compared to that of the laboratory environment) may be too high for accurate measurements of behavioral divergences between populations that stem from small genetic differences. Additionally, even when laboratory models are outbred, the generation of animals being used is still orders of magnitude higher than the generations of animals used here. For example, Sprague-Dawley rat colonies at Charles River were established in the 1990s, whereas the prairie voles used in this research are a maximum of eight generations from wild-caught. Therefore, any genetic differences between prairie vole colonies at different university laboratories would be minimal compared to those between vendors of established laboratory animal models.

Overall, our findings here provide tentative support for the idea that environmental factors influence behavior more than individual traits or early life environment of voles bred in laboratory colonies, although we did not measure genetic differences between our populations, nor did we quantify the effects of environmental conditions in this study. To address these limitations, future studies should build on this study by conducting laboratory assays of space use and behavioral traits (e.g., anxiety; social preferences), as well as quantify any genetic differences between colony populations prior to introduction into enclosures for experimental use (see Ophir et al., 2007; Rice et al., 2022 for examples). In addition, the sharing of animal breeding and maintenance protocols between laboratories with animal colonies may be a positive addition to the field—because prairie voles are initially bred from wild voles caught across the United States, the standardization of animal protocols may decrease any existing behavioral differences among colonies.

4.6 Acknowledgements

4.7 References

An, X. L., Zou, J. X., Wu, R. Y., Yang, Y., Tai, F. D., Zeng, S. Y., Zhang, X., Liu, E., & Broders, H. (2011). Strain and sex differences in anxiety-like and social behaviors in C57BL/6J and BALB/cJ mice. *Experimental animals*, *60*(2), 111-123. https://doi.org/10.1538/expanim.60.111.

Baíllo, A., & Chacón, J. E. (2021). Statistical outline of animal home ranges: an application of set estimation. In *Handbook of Statistics* (Vol. 44, pp. 3-37). Elsevier.

Bale, T. L., Abel, T., Akil, H., Carlezon Jr, W. A., Moghaddam, B., Nestler, E. J., Ressler, K. J., & Thompson, S. M. (2019). The critical importance of basic animal research for neuropsychiatric disorders. *Neuropsychopharmacology, 44*(8), 1349-1353. https://doi.org/10.1038/s41386-019-0405-9.

Barton, N. H., & Charlesworth, B. (1984). Genetic revolutions, founder effects, and speciation. Annual review of ecology and systematics, 15(1), 133-164.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Bell, M. A., & Foster, S. A. (1994). Introduction to the evolutionary biology of the threespine stickleback. *The evolutionary biology of the threespine stickleback*, 1-27.

Bivand, R., Pebesma, E., & Gomez-Rubio, V. (2013). *Applied spatial data analysis with R, Second edition*.

Boakes, R. (1977). Performance on learning to associate a stimulus with positive reinforcement. In: Davis H, Hurwits H (eds) *Operant-pavlovian interactions*. Lawrence Erlbaum Associates, Hillsdale, pp 67–97.

Bolker, J. A. (2019). Selection of models: evolution and the choice of species for translational research. Brain Behavior and Evolution, 93(2-3), 82-91. https://doi.org/10.1159/000500317.

Cairns, S. J., & Schwager, S. J. (1987). A comparison of association indices. *Animal Behaviour, 35*(5), 1454-1469. https://doi.org/10.1016/S0003-3472(87)80018-0.

Calenge, C., & Fortmann-Roe, c.f.S. (2023). adehabitatHR: Home Range Estimation. R package version 0.4.21, https://CRAN.R-project.org/package=adehabitatHR.

Cauchoix, M., & Chaine, A. S. (2016). How can we study the evolution of animal minds?. *Frontiers in Psychology*, *7*, 358. https://doi.org/10.3389/fpsyg.2016.00358.

Chitty, D. (1960). Population processes in the vole and their relevance to general theory. Canadian Journal of Zoology, 38(1), 99-113. https://doi.org/10.1139/z60-011.

Chitty, D. (1967). What Regulates Bird Population. *Ecology 48*(4), 698-701. https://doi.org/10.2307/1936523

Clemens, L. E., Jansson, E. K. H., Portal, E., Riess, O., & Nguyen, H. P. (2014). A behavioral comparison of the common laboratory rat strains Lister Hooded, Lewis, Fischer 344 and Wistar in an automated homecage system. *Genes, Brain and Behavior*, *13*(3), 305-321. https://doi.org/10.1111/gbb.12093.

Cochran, G. R., & Solomon, N. G. (2000). Effects of food supplementation on the social organization of prairie voles (Microtus ochrogaster). *Journal of Mammalogy, 81*(3), 746-757. https://doi.org/10.1644/1545-1542(2000)081%3C0746:EOFSOT%3E2.3.CO;2.

Desy, E. A., & Batzli, G. O. (1989). Effects of food availability and predation on prairie vole demography: a field experiment. *Ecology, 70*(2), 411-421. https://doi.org/10.2307/1937546.

Duckworth, R. A. (2009). The role of behavior in evolution: a search for mechanism. *Evolutionary ecology*, *23*, 513-531. https://doi.org/10.1007/s10682-008-9252-6.

Farine, D. R. (2013). Animal social network inference and permutations for ecologists in R using asnipe. Methods in Ecology and Evolution, 4(12), 1187-1194. https://doi.org/10.1111/2041-210X.12121.

Faure, A., Pittaras, E., Nosjean, A., Chabout, J., Cressant, A., & Granon, S. (2017). Social behaviors and acoustic vocalizations in different strains of mice. *Behavioural brain research*, *320*, 383-390. https://doi.org/10.1016/j.bbr.2016.11.003.

Festing, M. F. (2010). Inbred strains should replace outbred stocks in toxicology, safety testing, and drug development. *Toxicologic pathology*, *38*(5), 681-690. https://doi.org/10.1177/0192623310373776.

Fitzpatrick, C. J., Gopalakrishnan, S., Cogan, E. S., Yager, L. M., Meyer, P. J., Lovic, V., ... & Morrow, J. D. (2013). Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. *PloS one*, *8*(10), e75042. https://doi.org/10.1371/journal.pone.0075042.

Foster, S. A., & Endler, J. A. (Eds.). (1999). Geographic variation in behavior: perspectives on evolutionary mechanisms. Oxford University Press, USA.

Getz, L. L., Hofmann, J. E., McGuire, B., & Dolan III, T. W. (2001). Twenty-five years of population fluctuations of *Microtus ochrogaster* and *M. pennsylvanicus* in three habitats

in east-central Illinois. *Journal of Mammalogy, 82*, 22-34.
http://dx.doi.org/10.1093/jmammal/82.1.22.

Getz, L. L., McGuire, B., Pizzuto, T., Hofmann, J. E., & Frase, B. (1993). Social organization of
the prairie vole (*Microtus ochrogaster*). *Journal of Mammalogy, 74*(1), 44–58.
https://doi.org/10.2307/1381904.

Gotelli, N. J., & Ellison, A. M. (2004). A primer of ecological statistics (Vol. 1, pp. 1-640).
Sunderland: Sinauer Associates.

Hearst, E., & Jenkins, H. M. (1974). Sign tracking: the stimulus-reinforcer relation and directed
action. *Monograph of the Psychonomic Society*, Austin.

Hoppitt, W. J., & Farine, D. R. (2018). Association indices for quantifying social relationships:
how to deal with missing observations of individuals or groups. *Animal Behaviour, 136*,
227-238. https://doi.org/10.1016/j.anbehav.2017.08.029.

Khoo, S. Y. S., Uhrig, A., Samaha, A. N., & Chaudhri, N. (2022). Does vendor breeding colony
influence sign-and goal-tracking in Pavlovian conditioned approach? A preregistered
empirical replication. *Neuroanatomy and Behaviour, 4*(1), e46.
https://doi.org/10.35430/nab.2022.e46.

Krackow, S., Vannoni, E., Codita, A., Mohammed, A. H., Cirulli, F., Branchi, I., ... & Lipp, H.
P. (2010). Consistent behavioral phenotype differences between inbred mouse strains in
the IntelliCage. *Genes, brain and behavior*, *9*(7), 722-731. https://doi.org/10.1111/j.1601-
183X.2010.00606.x.

Krause, J., Croft, D. P., & James, R. (2007). Social network theory in the behavioural sciences:
potential applications. *Behavioral Ecology and Sociobiology*, *62*, 15-27.
https://doi.org/10.1007/s00265-007-0445-8.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in
Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 1–26.
https://doi.org/10.18637/jss.v082.i13.

Loos, M., Koopmans, B., Aarts, E., Maroteaux, G., Van Der Sluis, S., Neuro-BSIK Mouse
Phenomics Consortium, Verhage, M., & Smit, A. B. (2014). Sheltering behavior and
locomotor activity in 11 genetically diverse common inbred mouse strains using home-
cage monitoring. *PLoS One*, *9*(9), e108563.
https://doi.org/10.1371/journal.pone.0108563.

Loos, M., Koopmans, B., Aarts, E., Maroteaux, G., van der Sluis, S., Neuro-BSIK Mouse
Phenomics Consortium, ... & Smit, A. B. (2015). Within-strain variation in behavior
differs consistently between common inbred strains of mice. *Mammalian genome*, *26*,
348-354. https://doi.org/10.1007/s00335-015-9578-7.

Lott, D. F. (1984). Intraspecific variation in the social systems of wild vertebrates. *Behaviour,
88*(3-4), 266-325.

Mayr, E. (1963). Animal species and evolution. Harvard University Press.

Mitchell, D., Beatty, E. T., & Cox, P. K. (1977). Behavioral differences between two populations of wild rats: implications for domestication research. *Behavioral Biology, 19*(2), 206-216. https://doi.org/10.1016/S0091-6773(77)91494-8.

Mohr, C. O. (1947). Table of equivalent populations of North American small mammals. *The American Midland Naturalist*, *37*(1), 223-249. https://doi.org/10.2307/2421652.

Olsson, I. A. S., Nevison, C. M., Patterson-Kane, E. G., Sherwin, C. M., Van de Weerd, H. A., & Würbel, H. (2003). Understanding behaviour: the relevance of ethological approaches in laboratory animal science. *Applied Animal Behaviour Science*, *81*(3), 245-264. https://doi.org/10.1016/S0168-1591(02)00285-X.

Ophir, A. G., Phelps, S. M., Sorin, A. B., & Wolff, J. O. (2007). Morphological, genetic, and behavioral comparisons of two prairie vole populations in the field and laboratory. *Journal of Mammalogy*, *88*(4), 989-999.https://doi.org/10.1644/06-MAMM-A-250R.1.

Pebesma E, Bivand R (2005). Classes and methods for spatial data in R. *R News 5*(2), 9-13.

Pebesma, E., & Bivand, R. (2023). *Spatial Data Science: With Applications in R*. Chapman and Hall/CRC. https://doi.org/10.1201/9780429459016

Pebesma, E., 2018. Simple Features for R: Standardized Support for Spatial Vector Data. *The R Journal 10*(1), 439-446, https://doi.org/10.32614/RJ-2018-009.

Powell, R. A. (2000). Animal home ranges and territories and home range estimators. In *Research techniques in animal ecology: controversies and consequences*, 442, 65-110.

Powell, R. A., & Mitchell, M. S. (2012). What is a home range?. *Journal of mammalogy, 93*(4), 948-958. https://doi.org/10.1644/11-MAMM-S-177.1.

Psorakis, I., Voelkl, B., Garroway, C. J., Radersma, R., Aplin, L. M., Crates, R. A., ... & Sheldon, B. C. (2015). Inferring social structure from temporal data. *Behavioral Ecology and Sociobiology*, *69*, 857-866. https://doi.org/10.1007/s00265-015-1906-0.

Rice, M. A., Galindez, S. M., Garner, J. T., & Ophir, A. G. (2022). Effects of social and environmental contexts on multi-male mating and mixed paternity in socially monogamous female prairie voles. *Royal Society Open Science, 9*(10), 220298. https://doi.org/10.1098/rsos.220298.

Richter, S. H., Garner, J. P., Zipser, B., Lewejohann, L., Sachser, N., Touma, C., ... & Würbel, H. (2011). Effect of population heterogenization on the reproducibility of mouse behavior: a multi-laboratory study. *PLoS One, 6*(1), e16461. https://doi.org/10.1371/journal.pone.0016461.

Row, J. R., & Blouin-Demers, G. (2006). Kernels are not accurate estimators of home-range size for herpetofauna. *Copeia*, *2006*(4), 797-802. https://doi.org/10.1643/0045-8511(2006)6[797:KANAEO]2.0.CO;2.

Rowlingson B, Diggle P (2023). splancs: Spatial and Space-Time Point Pattern Analysis. R package version 2.01-44, https://CRAN.R-project.org/package=splancs.

Sokal, R. R. & Rohlf, F. H. (1995). *Biometry: the principles and practice of statistics in biological research. Third edition.* W. H. Freeman. New York, New York, USA. ISBN-13: 978-0716724117.

Solomon, N. G., & Keane, B. (2018). Dispatches from the field: sociality and reproductive success in prairie voles. *Animal Behaviour*, *143*, 193-203. https://doi.org/10.1016/j.anbehav.2018.07.001.

Solomon, N. G., Richmond, A. R., Harding, P. A., Fries, A., Jacquemin, S., Schaefer, R. L., Lucia, K.E., & Keane, B. (2009). Polymorphism at the avpr1a locus in male prairie voles correlated with genetic but not social monogamy in field populations. *Molecular Ecology, 18*(22), 4680-4695. https://doi.org/10.1111/j.1365-294X.2009.04361.x.

Sultana, R., Ogundele, O. M., & Lee, C. C. (2019). Contrasting characteristic behaviours among common laboratory mouse strains. *Royal Society Open Science, 6*(6), 190574. http://dx.doi.org/10.1098/rsos.190574.

Wahlsten, D., Metten, P., Phillips, T. J., Boehm, S. L., Burkhart-Kasch, S., Dorow, J., ... & Crabbe, J. C. (2003). Different data from different labs: lessons from studies of gene–environment interaction. *Journal of neurobiology*, *54*(1), 283-311. https://doi.org/10.1002/neu.10173.

Warton, D. I., & Hui, F. K. (2011). The arcsine is asinine: the analysis of proportions in ecology. Ecology, 92(1), 3-10. https://doi.org/10.1890/10-0340.1.

White, G. C., & Garrott, R. A. (1990). *Analysis of Wildlife Radiotracking Data*. Academic Press: San Diego , CA .

Wolff, J. O., & Dunlap, A. S. (2002). Multi-male mating, probability of conception, and litter size in the prairie vole (*Microtus ochrogaster*). *Behavioural Processes, 58*(1-2), 105-110. https://doi.org/10.1016/S0376-6357(02)00022-0.

Wolff, J., Mech, S., Dunlap, A., & Hodges, K. (2002). Multi-male mating by paired and unpaired female prairie voles (*Microtus ochrogaster*). *Behaviour, 139*(9), 1147-1160. https://doi.org/10.1163/15685390260437308.

Yi, P., & Li, L. (2012). The germfree murine animal: an important animal model for research on the relationship between gut microbiota and the host. *Veterinary Microbiology*, *157*(1-2), 1-7. https://doi.org/10.1016/j.vetmic.2011.10.024.

Zar, J. H. (1998). *Biostatistical analysis. Fourth edition.* Prentice Hall. Englewood Cliffs, New Jersey, USA. ISBN 13: 9780130815422.

4.8 Tables and Figures

Figure 4.1: Relationship between weight (g) and age (days) of the founding groups of prairie voles (Burkett in blue, Grippo in pink, and Solomon-Keane in orange), prior to their release into the semi-natural enclosures. Burkett voles were heavier for their age than Grippo (p = 0.009) or Solomon-Keane voles (p = 0.004).
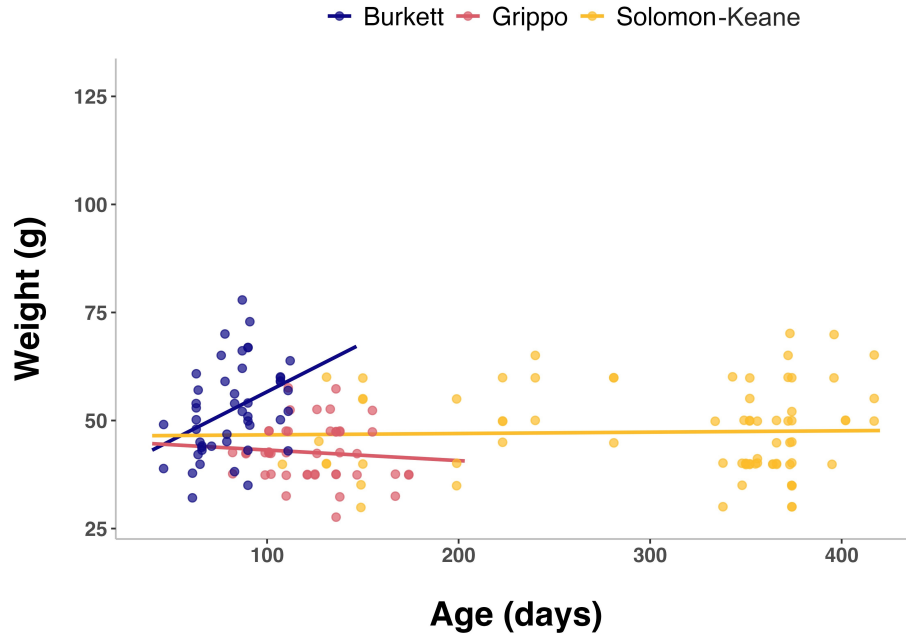

Figure 4.2: Distribution of voles from each laboratory colony that survived to the end of the study (in orange) and did not survive until the end of the study (in dark pink). There were no significant differences in the proportion of voles that survived until the end of the study among the voles bred in the Burkett (left), Grippo (middle), and Solomon-Keane (right) laboratories (p = 0.115). Within the vole populations, 27% of Burkett voles, 23% of Grippo voles, and 13% of Solomon-Keane voles survived until the end of the study period.
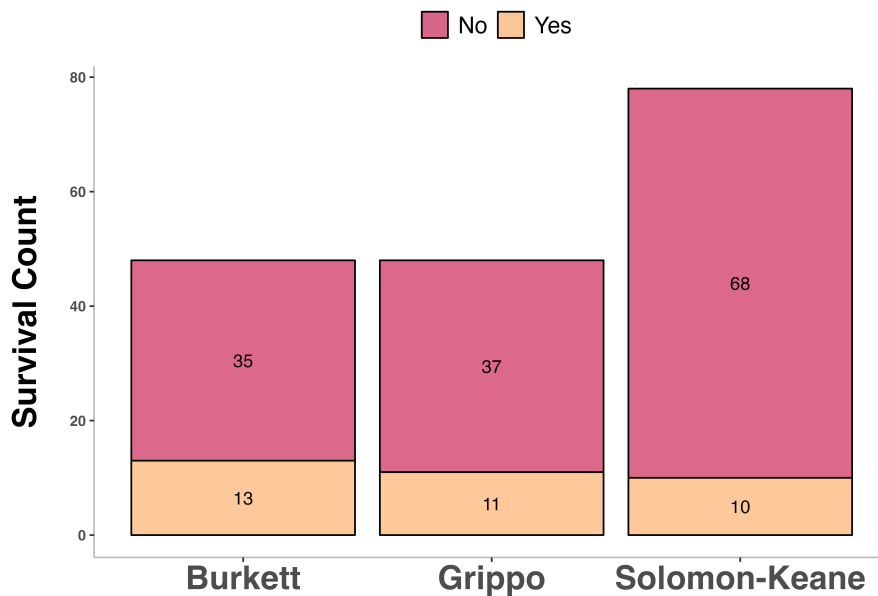
Figure 4.3: Diagram of home ranges, calculated as convex hulls by live trapping data, in each of eight enclosures. Enclosures labelled "Grippo" were populated with voles bred in the Grippo Laboratory in 2019; enclosures labelled "Solomon-Keane" were populated with voles bred in the Solomon-Keane Laboratory in 2017; and enclosures labelled "Burkett" were populated with voles bred in the Burkett Laboratory in 2023. Each box represents an enclosure, with the x and y coordinates in meters. The number of voles established in each enclosure is indicated in the top right corner of each box.
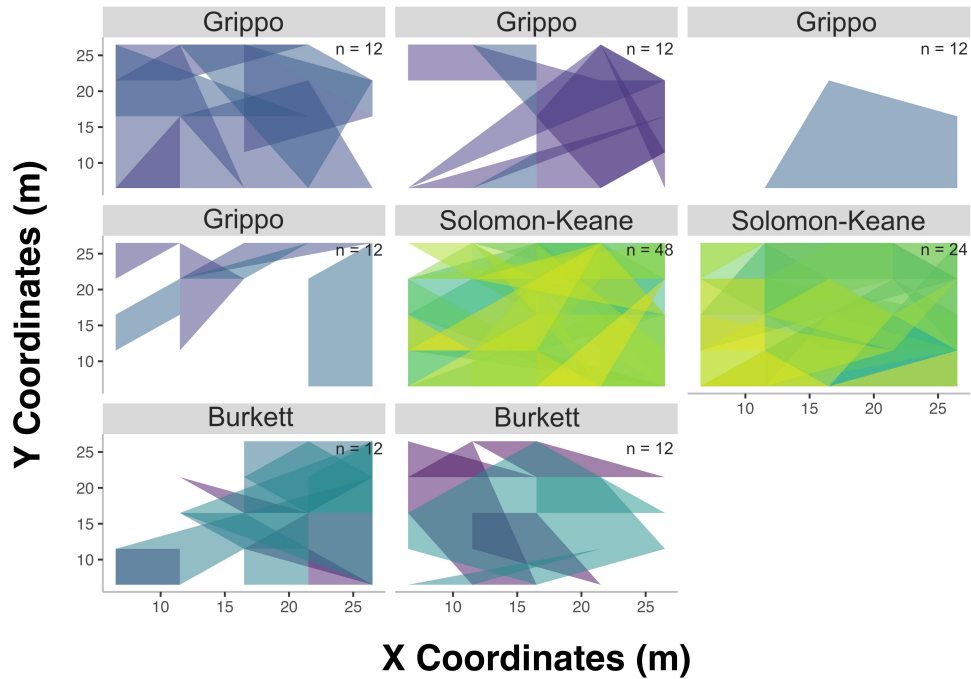

Figure 4.4: Comparison of home range areas among voles bred in the Burkett (far left, blue), Grippo (middle, purple), and Solomon (right, yellow) laboratories, measured using at least three unique trapping locations. There were no significant differences in home range areas among the three founding populations ($p = 0.4, 0.2$, respectively).
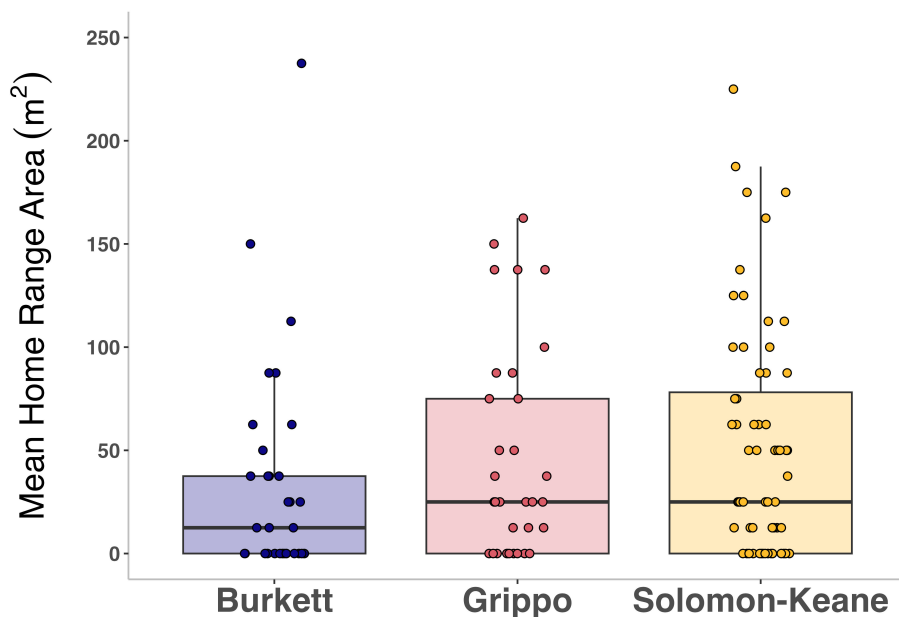
Figure 4.5: Count of residents (teal) and wanderers (blue) among voles bred in the Burkett (left), Grippo (middle), and Solomon-Keane (right) laboratories. There were no significant differences in the proportion of residents and wanderers among the three founding populations ($p = 0.09$). Resident and wandering tactics were assigned using degree of home range overlap among individuals (methods detailed in ***4.3.6 Statistical Analyses***).
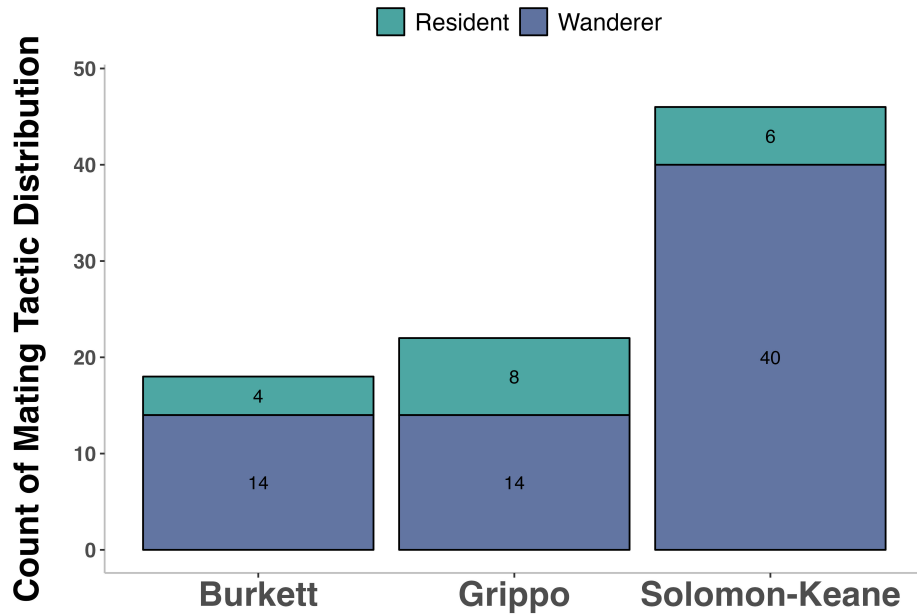

Figure 4.6: Comparison of home range areas among voles bred in the Grippo (left, purple), and Solomon (right, yellow) laboratories, measured using detections at three or more RFID antennas. Voles bred in the Grippo Laboratory had higher average home range areas than voles bred in the Solomon-Keane Laboratory ($p < 0.001$).
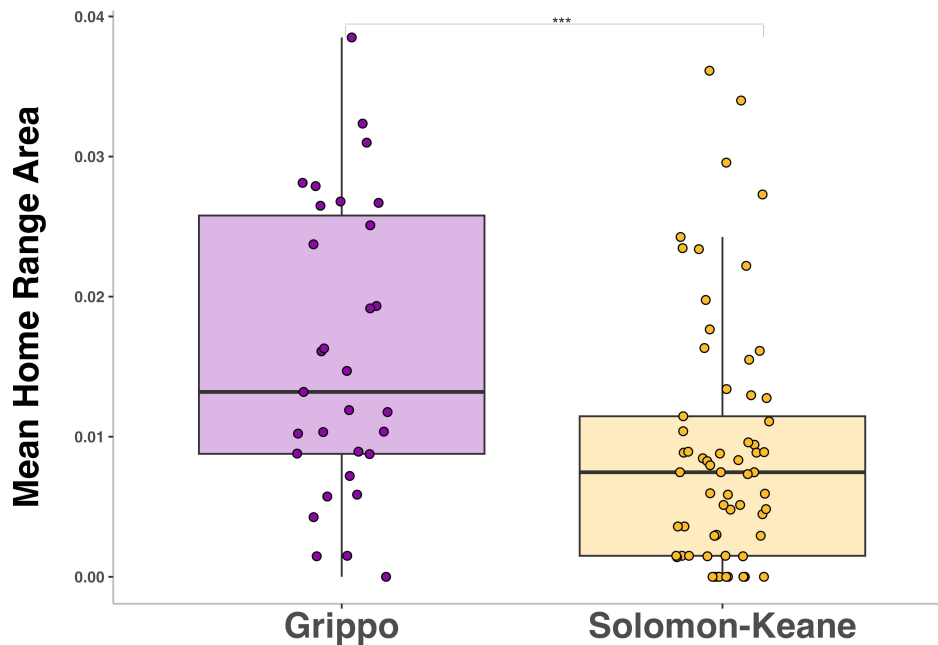
Figure 4.7: Unweighted degree summarized from social networks constructed using spatial-temporal overlap from live trapping data. Voles bred in the Solomon-Keane Laboratory (right, yellow) had higher average unweighted degree than voles bred in the Burkett (left, blue) or Grippo (middle, purple) laboratories ($p < 0.0001$).
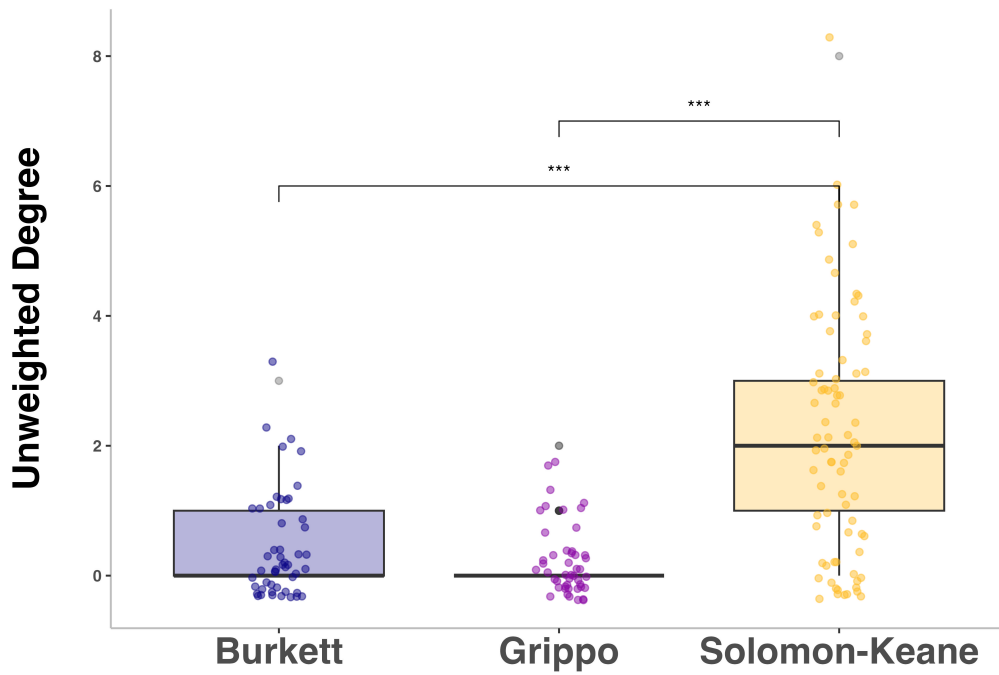

Figure 4.8: Degree weight summarized from social networks constructed using spatial-temporal overlap from live trapping data. Voles bred in the Solomon-Keane Laboratory (right, yellow) had higher average degree weight than voles bred in the Burkett (left, blue) or Grippo (middle, purple) laboratories ($p < 0.0001$).
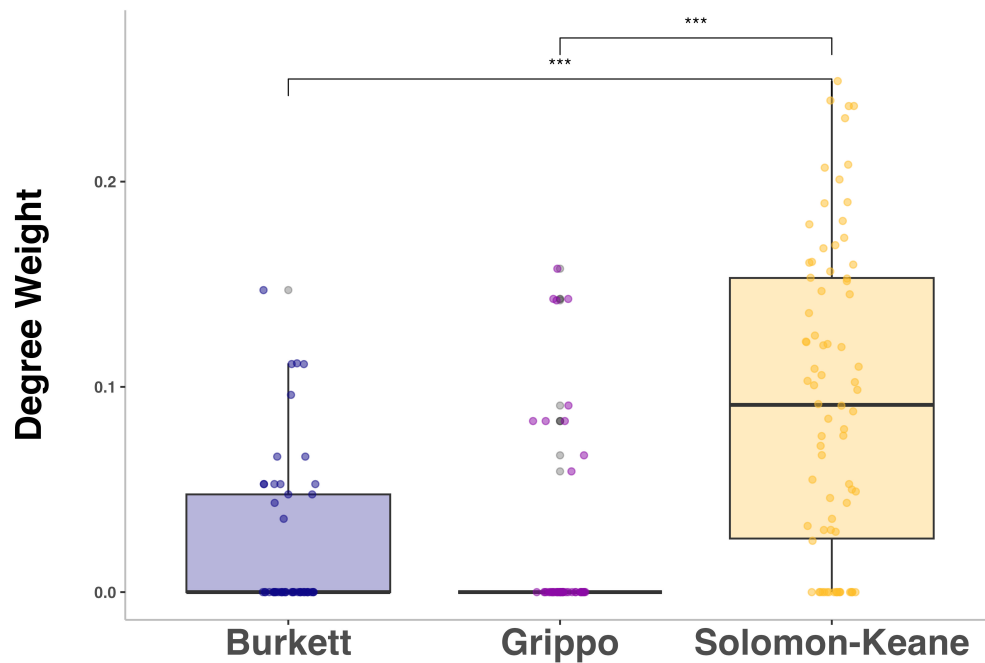
Figure 4.9: Unweighted degree summarized from social networks constructed using spatial-temporal overlap from RFID data. Voles bred in the Solomon-Keane Laboratory (right, yellow) had higher average unweighted degree than voles bred in the Grippo (left, purple) Laboratory ($p < 0.0001$).

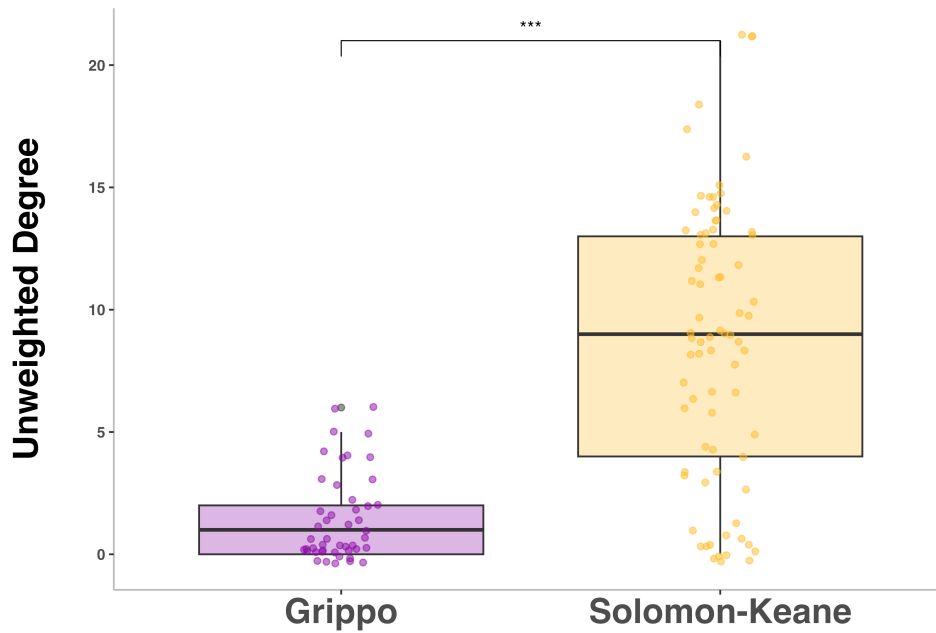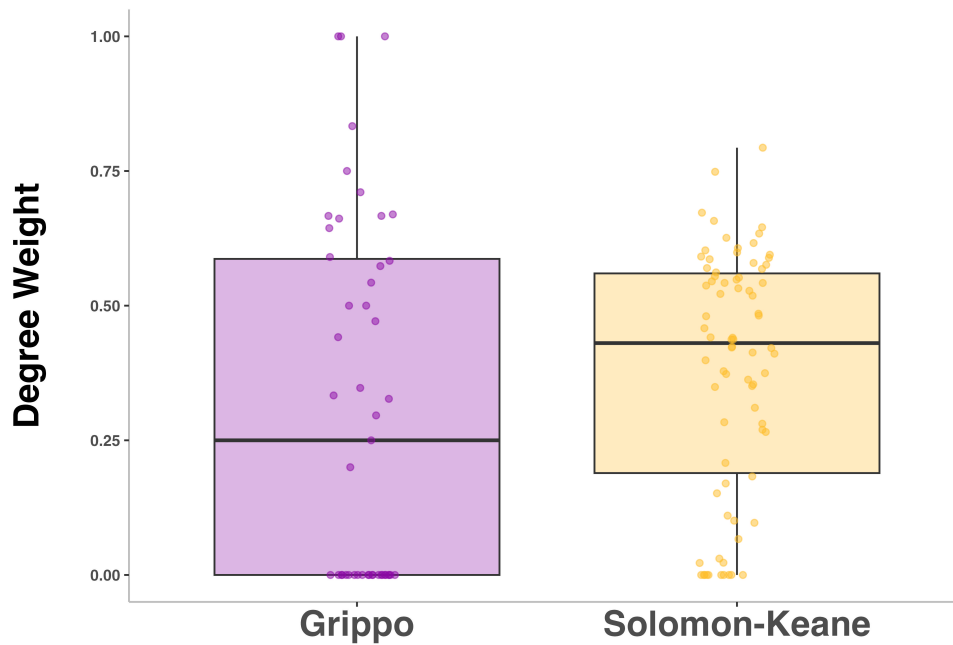**Figure 4.10**: Degree weight summarized from social networks constructed using spatial-temporal overlap from RFID data. There were no significant differences in degree weight between vole bred in the Grippo (left, purple) and Solomon (right, yellow) laboratories ($p > 0.05$).

Chapter 5 Conclusion

5.1 Overview

In this dissertation, I tested a new method for the recording of fine scale social behavior, provided clarity to our understanding of the factors that affect social affiliation, and determined whether laboratory breeding affects prairie vole behavior in natural settings. My first study compared three methods for measuring fine scale social behavior in small animals and demonstrated the proof of concept for the use of proximity loggers on small, highly social, and semi-fossorial mammals. Measuring questions of fine-scale social behavior in small mammals has been previously impossible; therefore, by addressing the issues of size, weight, and durability in proximity logger packaging, I tested a groundbreaking method that opens up a myriad of possibilities for future studies of social behavior (Prange et al., 2006).

During my second study, I directly compared three existing hypotheses to explain seasonal variation in affiliative behavior in a prairie vole model, integrating concepts from evolutionary biology, ecology, mammalogy, and decades of prairie vole research. Although studies have tested individual hypotheses regarding seasonal patterns of affiliative behavior in *Microtus* species (e.g., Getz et al., 1990; Madison et al., 1984; Lucia et al., 2008), no study has directly compared these competing hypotheses. We found that when controlling for aspects of the social and physical environment, as well as individual traits, we were able to narrow down the factors that most reliably predict prairie vole social behavior.

Finally, during my third study, I compared several aspects of behavior and demography among populations founded from a variety of laboratory breeding colonies. There is a large knowledge base regarding behavioral variability present in experiments with laboratory animals; however, this line of research has seldom been expanded into studies of laboratory animals introduced into semi-natural conditions. Therefore, I explored the potential for behavioral divergences in generations of laboratory-bred prairie voles, descended from wild-caught voles in Illinois. Explicitly investigating these questions in nature, with voles navigating survival and reproductive challenges, provides context to the many prairie vole studies previously conducted in semi-natural enclosures, which have resulted in highly varied results (e.g., Ophir et al., 2008; Solomon & Jacquot, 2002).

Through the development of novel methods for measuring fine scale behavior in small mammals, along with the testing of hypothesis to explain variation both within and among independent populations of animals, this dissertation contributes to the forward movement of the field of mammalogy, considers past theories of evolution and social behavior, and provides recommendations for innovative study designs. Below, I discuss the specifics of each of my dissertation studies and their implications for the field.

5.1.1 What are the most suitable methods for measuring fine scale social behavior?

In **Chapter 2**, I aimed to identify a suitable method for the measurement of fine scale social behavior in small mammals under natural conditions. We tested a developing model of proximity logger, which detected interactions among animals at close range. Ultimately, the proximity loggers were able to record social behavior data at unprecedented resolutions beyond the data estimated from spatial-temporal overlap via more traditional live trapping and RFID data. Proximity loggers produced many more observations across much shorter timescales. We

also found that the social networks constructed from trapping data predicted the social networks constructed from proximity logger data. Therefore, although there remains a high level of unexplained variation in patterns of social behavior among prairie voles, we concluded that proximity loggers record similar patterns of behavior to traditional trapping methods. Overall, proximity loggers are suitable for answering questions about fine scale social behavior and provide a promising new option to expand our understanding of social behavior in small, nonhuman animals.

5.1.2 What factors most reliably predict close social contact?

In **Chapter 3**, we found that adult sex ratio most reliably predicts the number of close social contacts (i.e., unweighted degree) among prairie voles, while adult sex ratio and adult population density reliably predict the strength of these contacts (i.e., degree weight). Rather than being driven by aspects of the abiotic environment or individual traits, demographical facets of the social environment were most closely associated with measures of social behavior. Importantly, these findings are consistent with leading hypotheses regarding the influence of adult sex ratio and adult population density on levels of sociality. Evolutionary theory predicts that male-biased sex ratios should favor mate guarding and female-biased sex ratios should favor promiscuous mating that benefits both sexes (Bateman, 1940; Emlen & Oring, 1977; Shuster & Wade, 2003). We found tentative support for both of these hypotheses, as social connections were strongest and fewest in male-biased sex ratios and social connections were both abundant and highly weighted in female-biased sex ratios. Additionally, the Polymorphic behavior hypothesis regarding the maintenance of population density cycles in small mammals posits that individuals in high density conditions should be highly aggressive while individuals in low density conditions should be highly affiliative (Chitty, 1960, 1967; Edwards et al., 2021). We

found a quadratic relationship between degree weight and adult population density, such that degree weight was highest under low-density and high-density conditions; although we cannot confirm the affective valence of the social contacts we detected, the polymorphic behavior hypothesis has potential to add explanatory value to our results.

5.1.3 Does the breeding history and early life laboratory environment matter for social behavior in natural contexts?

In **Chapter 4**, we compared social behavior in semi-natural enclosures among sets of founding voles acquired from four laboratory breeding colonies. We found quantifiable differences in body weight and age among our founding populations; however, we found limited evidence that these differences were associated with significant behavioral divergences. Instead, we applied our results from Chapter 3 to speculate that observed differences in home range area and number of social contacts may be related to differences in aspects of the social environment such as population density that we could not fully account for in our statistical analyses. Together, these results suggest that variation in gene pool, laboratory breeding protocols, or individual traits of voles upon release into enclosures do not have a significant effect on the outcomes of field studies.

5.2 Reflections on Future Directions

There are nearly unlimited future directions that I could suggest from my work in this dissertation. To improve upon the methods reported here for the use of proximity loggers in small mammals, future researchers should apply proximity loggers to all conspecific members of a population and document the data collected on a longer time scale (e.g., months). Additionally, the application of proximity loggers to all conspecifics in a population would allow for the

development of high-resolution, dynamic social networks at an unprecedented scale. These methods could be used to gain a detailed understanding of space use, burrow use, parental care, group foraging behavior, disease spread, and the establishment of mating tactics, to name a few.

The combination use of proximity loggers and experimental methods could increase our understanding of social behavior, as well—for example, future experiments should manipulate features of the social environment such as adult sex ratio, adult population density, and juvenile mortality rates, and use proximity loggers to quantify group formation. The combination of proximity logger use with measures of partner preference (i.e., PPT; e.g., Sabol et al., 2018), stress physiology, and health would also offer insights into pair bonding in natural settings. Future experiments could also measure the formation of pair bonds between monogamous pairs of conspecifics, disrupt existing pair bonds by separating monogamous social partners, and observe the behavioral effects and physiological consequences on both the separated male and separated female.

Future work regarding potential founding effects from behavioral divergences in the laboratory animals used to establish semi-natural populations should directly measure genetic markers and aspects of behavior such as space use, memory, and social motivation, prior to introduction into the enclosures (e.g., Ophir et al., 2007; Rice et al., 2022). In this way, behavior in semi-natural settings can be directly mapped back to any genetic divergence between populations and pre-existing behavioral traits, if they exist.

5.3 Concluding Remarks

In this dissertation, I focused on developing methods for measuring fine scale social behavior in small mammals (**Chapter 2**), determining the most reliable predictors of close social association (**Chapter 3**), and quantifying any behavioral variation in natural behavior among

prairie voles from different immediate genetic and environmental backgrounds (**Chapter 4**). All

chapters improve researchers' ability to research and understand social behavior, including the

causality and fitness consequences of behaviors involved in pair bonding and mating. Future

research should take advantage of proximity loggers to investigate questions of fine-scale social

behavior and its interactions with environment, physiology, and genetics. Ultimately, this

dissertation contributes to the body of knowledge regarding influences on social behavior and the

ways that they can be studied, lending insight to the development of future studies that

investigate the evolution and maintenance of social behavior from functional and mechanistic

perspectives, at a greater depth than has been previously possible.

5.4 References

Bateman, A. J. (1948). Intra-sexual selection in *Drosophila*. *Heredity, 2*, 349–368. https://doi.org/10.1038/hdy.1948.21.

Emlen, S. T., & Oring, L. W. (1977). Ecology, sexual selection, and the evolution of mating systems. *Science, 197*, 215-223. https://doi.org/10.1126/science.327542.

Shuster, S. M., & Wade, M. J. (2003). *Mating systems and mating strategies.* Princeton University Press. ISBN 0-691-04931-9.

Chitty, D. (1960). Population processes in the vole and their relevance to general theory. Canadian Journal of Zoology, 38(1), 99-113. https://doi.org/10.1139/z60-011.

Chitty, D. (1967). What Regulates Bird Population. *Ecology 48*(4), 698-701. https://doi.org/10.2307/1936523

Edwards, P. D., Frenette-Ling, C., Palme, R., & Boonstra, R. (2021). A mechanism for population self-regulation: Social density suppresses GnRH expression and reduces reproductivity in voles. *Journal of Animal Ecology, 90*(4), 784-795. https://doi.org/10.1111/1365-2656.13430.

Sabol, A. C., Solomon, N. G., & Dantzer, B. (2018). How to study socially monogamous behavior in secretive animals? Using social network analyses and automated tracking systems to study the social behavior of prairie voles. *Frontiers in Ecology and Evolution, 6*, 178. https://doi.org/10.3389/fevo.2018.00178.

Ophir, A. G., Phelps, S. M., Sorin, A. B., & Wolff, J. O. (2007). Morphological, genetic, and behavioral comparisons of two prairie vole populations in the field and laboratory. *Journal of Mammalogy*, *88*(4), 989-999.https://doi.org/10.1644/06-MAMM-A-250R.1.

Rice, M. A., Galindez, S. M., Garner, J. T., & Ophir, A. G. (2022). Effects of social and environmental contexts on multi-male mating and mixed paternity in socially monogamous female prairie voles. *Royal Society Open Science, 9*(10), 220298. https://doi.org/10.1098/rsos.2