Behavioral Heterogeneity and Disease Dynamics in House Finches

(*Haemorhous mexicanus*)

Sahnzi Chow Moyers

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Biological Sciences

Dana M. Hawley, Chair

Lisa K. Belden

Jeffrey R. Walters

William A. Hopkins

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Behavioral Heterogeneity and Disease Dynamics in House Finches

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**ABSTRACT**

Infectious disease is a ubiquitous aspect of life on earth; however, parasites and pathogens are not distributed equally among individual hosts. Due to its ability to shape the way that individuals interact with other potential hosts and the environment, behavior is one of the most salient ways through which host biology varies in the context of disease. Variation in animal behavior can impact both transmission and the extent of a host’s pathogen acquisition, and thus can have important consequences for infectious disease dynamics. Additionally, in this world of rapid urbanization where landscapes and wildlife resources are being altered, it is important to understand the ways in which human activity impact wildlife behavior, and in turn, disease dynamics. Here, we used both observational and experimental studies in field and laboratory settings to investigate the relationships among host behavior and physiology, anthropogenic food sources, and disease transmission in a natural host-pathogen system. First, we examined the relationship between house finch (*Haemorhous mexicanus*) stress physiology, exploratory behaviors, and social behaviors in the wild. We provided evidence that more exploratory house finches interact with more individuals in the wild, and have higher baseline concentrations of circulating stress hormones. Next, we found evidence that the amount of time spent on bird feeders drives both the acquisition and transmission of the bacterial pathogen *Mycoplasma gallisepticum* (Mg), indicating that variation in host foraging behavior has
important transmission consequences in this system. Lastly, we found that the density of bird feeders available to house finches predicts the extent of Mg transmission in captivity. Taken together, these results highlight the important role that behavioral heterogeneity can play in the acquisition and spread of pathogens, as well as the potential impacts of human behavior on wildlife disease dynamics. Future work should seek to identify specific physiological mechanisms driving Mg acquisition and transmission as they relate to variation in host behavior, and the ways in which bird feeders impact disease-relevant behaviors in the wild.
Behavioral Heterogeneity and Disease Dynamics in House Finches

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**GENERAL AUDIENCE ABSTRACT**

Infectious disease impacts almost every living creature on earth; however, some individuals are more likely to become sick and spread disease than others. Animal behavior can strongly influence disease dynamics due to its ability to shape the way that individuals interact with one another and the environment. Behavior can impact an individual’s likelihood of both acquiring and spreading disease, and thus can have important consequences for disease outbreaks. Additionally, as urban areas are expanding, it is important to understand the ways in which human activity impact wildlife behavior, and in turn, disease dynamics. Through both laboratory and field studies, we investigate the relationships among host behavior and physiology, human-related food sources, and disease transmission in a natural wildlife disease system. First, we examined the relationships between stress hormones, exploratory behaviors, and social behaviors of house finches, a common songbird. We provided evidence that more exploratory house finches interact with more individuals in the wild, and have higher concentrations of stress hormones. Next, we found evidence that the amount of time that house finches spend on bird feeders drives both the likelihood of acquiring and spreading conjunctivitis (=pink eye). This means that certain individuals are more likely to get sick and pass the disease on to others than other individuals are. Lastly, we found that when the density of bird feeders available to house finches is high, we see more disease transmission. Taken together, these
results highlight the important role that variation in behavior can play in acquiring and spreading disease, as well as the potential impacts of human behavior on wildlife health.
Dedication

I dedicate this work to all of the family, friends, collaborators, mentors, and birds who have inspired and assisted me along the way.
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Attributions

Dr. Dana M. Hawley, Associate Professor, Department of Biological Sciences, Virginia Tech, Blacksburg, Virginia. Dr. Hawley was my advisor and committee chair. She assisted in the design, execution, and statistical analyses of all of the studies presented in this dissertation, and is a coauthor on all of the manuscripts in this dissertation (Chapters, II, III, and IV).

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Chapter I: Introduction

From blue whales to bacteria, no organisms on earth are free from the ecological and evolutionary pressures of infectious diseases. Parasitism is estimated to be the most common life strategy on Earth (Dobson et al., 2008; Poulin and Morand, 2000), and while most organisms are parasitized in some way, these parasites and pathogens are not evenly distributed across individual hosts (e.g. Shaw et al., 1998). Aggregation of parasites within a small proportion of hosts is so common that Poulin (2007) named parasite aggregation as one of the “general laws” in parasite ecology. Additionally, a minority of the host population (20%) frequently drive the majority of parasite and pathogen transmission (80%) (Woolhouse et al., 1997). Although epidemiological models are commonly used to assess the potential spread of a disease, these models often oversimplify the intricacies of transmission dynamics, and underestimate the role of host heterogeneity in infection (Anderson and May, 1979). Assessing the origin of heterogeneities in infection within populations or social groups is an integral part of understanding disease dynamics on a larger scale, and can help us to better predict the trajectories of disease outbreaks for management and conservation purposes.

Models indicate that the extent of heterogeneity among individuals in transmission can largely determine the probability and severity of an epidemic in a host population (Lloyd-Smith et al., 2005). In epidemiological models, the basic reproductive number (hereafter $R_0$) of a pathogen is used to predict whether or not a disease will persist within a population, and the severity of resulting epidemics (Box 1). Within a susceptible population, the presence of individuals who contribute disproportionately to the acquisition and/or spread of disease can shape the likelihood and intensity of epidemics.

Box 1. Basic Reproductive Number ($R_0$): the mean number of secondary infections caused by one infected individual within a wholly susceptible population (Lloyd-Smith et al. 2005).
Individuals that have a disproportionally high capacity to acquire and transmit pathogens have been called “super-receivers” and “super-spreaders”, respectively (Hamede et al., 2013; Shen et al., 2004). One of the most important sources of heterogeneity in these traits is host behavior, which can influence exposure to infectious agents, susceptibility to disease once exposed, and the likelihood of transmitting the pathogen to other hosts. All three of the aforementioned factors largely determine the likelihood of an infectious disease invading and persisting within a population.

**Behavioral heterogeneity and pathogen acquisition**

In order for the transmission of an infectious disease to occur, a susceptible host must come into contact with an infectious agent via environmental exposure or direct interaction with infected individuals. The probability of either mode of exposure occurring will be strongly influenced by host behavior. At the species level, variation in social organization predicts infection prevalence, indicating the importance of host behavior to pathogen acquisition. For example, Ezenwa (2004) found that for male gazelle, gregarious species have higher parasite burdens than territorial (solitary) species. Within a species, for diseases that follow a density-dependent transmission pattern, intraspecific contact rates increase with host density, increasing the probability of transmission between an infected individual and a susceptible individual (Anderson and May, 1979). This positive correlation between group size and the prevalence of directly-transmitted disease has been documented in many species (Brown and Brown, 1986; Cote and Poulin, 1995; Davies et al., 1991; Moore et al., 1988; Nunn et al., 2003).

While previous studies highlight how behavior affects incidence of disease at the species level or group level, inter-individual differences in behavior within a group as it relates to
exposure to pathogens has only recently attracted attention and study. Inter-individual variation in behaviors that influence direct- or indirect-transmission have been linked to variation in exposure to parasites and pathogens in several wildlife host species, with most studies focusing on social behaviors, such as social status (MacIntosh et al., 2012), social network connectivity (Fenner et al., 2011; Godfrey et al., 2010, 2009), grooming (Drewe, 2010), and aggression (Drewe, 2010; Hamede et al., 2013). While variation in social behaviors is important for pathogens that are directly transmitted, behaviors such as space use can influence host exposure to infectious agents via environmental or short-term indirect transmission (Boyer et al., 2010; Weber et al., 2012). Thus, the host behaviors most relevant for variation in exposure will depend on the transmission mode of the pathogen.

One way of describing variation in animal behavior is through animal personalities, or consistent inter-individual differences in behavior (Réale et al., 2010; Sih et al., 2004). One of the most central axes of animal personality is exploratory behavior, wherein individuals are placed on a behavioral spectrum that ranges from “bold” (very exploratory) to “shy” (neophobic, or less exploratory) based on their responses to novel environments, objects, and/or conspecifics (e.g. Dingemanse et al., 2007; Drent et al., 2002; Sih et al., 2004; Verbeek and Drent, 1994). Exploratory tendencies can influence the ways in which an individual interacts with conspecifics, other species, and the environment (e.g. Aplin et al., 2013; Boyer et al., 2010; Favati et al., 2014; Pruitt and Modlmeier, 2015), and all of these could potentially impact disease dynamics. To date, several studies have shown links between personality traits, exposure to infectious agents (e.g. Boyer et al., 2010; Gyuris et al., 2016), and disease transmission (e.g. Keiser et al., 2016).
Physiological Susceptibility Upon Exposure to Pathogens

A host’s physiological susceptibility to parasites and pathogens upon exposure is an intricate interplay between many different endogenous and exogenous factors. Some of these factors include genetics, sex, body condition, host nutrition, immunocompetence, and hormonal state. Host behavior and social dynamics of group-living organisms can interact with several of the above-mentioned factors in ways that may affect an individual’s susceptibility to disease (Fairbanks and Hawley, 2012). In some cases, the same behaviors that mediate among-individual variation in exposure to pathogens (e.g. social dominance; Fairbanks and Hawley, 2012) are also associated with physiological mechanisms that either augment or decrease susceptibility once exposed (e.g. social dominance; Habig and Archie, 2015). For instance, a socially dominant individual may have greater foraging success and thus have both better nutrition and body condition, potentially increasing resistance to parasites and pathogens. Additionally, many studies have found links between testosterone, behavior, and immune response (e.g. Ezenwa et al., 2012). This behaviorally-mediated covariation in exposure and susceptibility among individuals can, in some cases, allow or prevent pathogen invasion in a population (Hawley et al., 2011). Currently, explicit links between inherent and predictable differences in animal behavior and physiological susceptibility to disease are needed. Because exploratory behavior can influence both exposure (e.g. Boyer et al., 2010; Gyuris et al., 2016), and susceptibility to infection (Koprivnikar et al., 2012), exploration behavior may be an example of a behavior that can have effects on both the likelihood of exposure to pathogens (via behavioral differences) and susceptibility to infection once exposed (via physiological differences).
Behavioral heterogeneity and super-spreading

Upon exposure to an infectious agent and subsequent infection, behavioral heterogeneity can influence the likelihood that an infectious individual will transmit disease to other hosts. The term super-spreader, which refers to an individual who transmits disease to an unusually high number of individuals, was coined after the 2003 severe acute respiratory syndrome (SARS) outbreak (Lloyd-Smith et al., 2005; Sherertz et al., 2001). Perhaps the most famous super-spreader of all time, an Irish cook named Mary Mallon, is thought to have infected more than 54 people with typhoid fever in the early 1900’s (Fairbanks and Hawley, 2012). As a consequence of her reputation, Mary was branded with the memorably blunt nickname “Typhoid Mary.”

Currently the presence of super-spreaders has not been documented for many host-pathogen systems, and thus the origins of super-spreading events, as well as the characteristics of super-spreaders are poorly understood. It remains unclear whether individuals become super-spreaders because they have higher than usual contact rates with susceptible individuals, are more infectious than other infected individuals, or a combination of the two. Furthermore, there have been no experiments to test how the presence of super-spreaders influences the spread of disease within a population, or to link super-spreading to predictable behavioral traits.

Anthropogenic influences on host behavior and disease dynamics

One of the main ways that human activity influences disease dynamics in wildlife is through resource provisioning, which alters disease dynamics in several host-pathogen systems (reviewed in Becker et al., 2015). Anthropogenic food supplementation can influence wildlife disease dynamics through increased density-dependent transmission due to changes in
demography or altered contact rates among hosts (Miller et al., 2003), altered contact rates between susceptible hosts and environmental sources of infectious agents (Cross et al., 2007), and changes in host condition and susceptibility (Lane et al., 2011). Overall, while food supplementation has been broadly linked to disease outbreaks in several wildlife host taxa, many of these studies have been observational in nature, and experimental studies examining how resource provisioning alters disease transmission dynamics are needed.

Current study

In this dissertation I explored topics of behavioral heterogeneity and physiological correlates, pathogen acquisition and transmission, and anthropogenic impacts on behavior and disease dynamics in an ecologically relevant host-pathogen system. I used observational and experimental studies, in field and laboratory contexts to address the following research objectives:

1. Investigate the relationships between stress physiology, heterogeneity in exploratory behavior, sociality, and foraging behaviors in house finches (Chapter II).
2. Examine behaviors likely to influence disease acquisition and transmission in the house finch – *Mycoplasma gallisepticum* host-pathogen system (Chapter III).
3. Investigate the role of anthropogenic resource provisioning on the disease dynamics of house finches (Chapter IV).

Host: House Finches (*Haemorhous mexicanus*)

House finches are small passerine birds that are now found throughout North America (Hill, 1993). Native to the western United State, house finches were introduced to the eastern
United States in the 1940s following efforts to sell them as domesticated pets (Fischer et al., 1997). They are relatively gregarious birds that frequently visit bird feeders in suburban and urban areas. During the non-breeding season, house finches aggregate into fluid flocks (Hill, 1993). Social dominance hierarchies are formed within these flocks, and dominance interactions are often observed when house finches forage as a group (Thompson, 1960a).

**Study Pathogen and Disease**

*Mycoplasma gallisepticum* (hereafter Mg) is a bacterial pathogen that belongs in the class Mollicutes. One notable morphological characteristic of mycoplasmas is that they lack cell walls (Madigan *et al.* 2009), which precludes persistence outside of the host for long periods of time. A novel strain of Mg, which was previously only known to affect poultry, was first detected in house finches (*Haemorhous mexicanus*) in 1994, and spread rapidly throughout the eastern population of house finches (Ley *et al.* 1996, Fischer *et al.* 1997). In house finches, Mg infection causes mycoplasmal conjunctivitis, resulting in swelling and inflammation of ocular tissues, as well as severe ocular discharge, that can significantly impair a bird’s vision. Since its emergence, Mg has caused annual winter epidemics of mycoplasmal conjunctivitis in house finch populations throughout the eastern United States. The surge of Mg infection prevalence during the winter is strongly associated with the flocking behavior that house finches exhibit during the non-breeding season (Altizer *et al.* 2004). While mycoplasmal conjunctivitis itself is not a lethal disease in captivity, there is significant indirect mortality in the wild: Mg reduces overwinter survival rates in infected individuals (Faustino *et al.* 2004), and has caused substantial declines in house finch populations (Hochachka and Dhondt, 2000).
Mg has a 14-day infectious period in house finches, during which it can be spread via multiple modes of transmission (Dhondt et al. 2008). In poultry, Mg spreads via direct contact, airborne droplets and dust, as well as vertically (Fischer et al. 1997). In house finches, there are two primary modes of Mg transmission: direct contact, and short-term indirect contact on bird feeders (Dhondt et al. 2008). During the non-breeding season when Mg prevalence peaks, the primary source of direct contacts among individuals is through agonistic interactions (Thompson, 1960b). Therefore, the extent of intraspecific interactions at bird feeders, where both direct and indirect contact occurs in this system, is particularly relevant for understanding individual variation in exposure and transmission in this system.

Behaviors that underlie variation in contact rate, both among house finches and with environmental sources of Mg (i.e. bird feeders), along with their implications for disease dynamics have not yet been examined. This system offers a unique opportunity to combine field and lab data to understand a) whether exploratory behaviors are linked to physiological and behavioral correlates in the field; b) how social and feeding behaviors influence the likelihood of acquiring and spreading pathogens to susceptible flockmates; and c) the implications of anthropogenic resource provisioning on behavior and disease dynamics in backyard birds.
References


Fairbanks, B., Hawley, D., 2012. Interactions between host social behavior, physiology, and


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Chapter II: Exploratory behavior is linked to stress physiology and social network centrality in free-living house finches (*Haemorhous mexicanus*)

Co-authors: James S. Adelman, Damien R. Farine, Ignacio T. Moore, Dana M. Hawley

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Abstract

Animal personality can shape the ways that individuals interact with the environment and conspecifics, and has been proposed to influence population-level processes. We investigated the relationships between stress physiology, exploratory behavior (one aspect of animal personality), sociality, and foraging behaviors in wild house finches (*Haemorhous mexicanus*). We conducted novel environment assays on house finches after collecting samples of baseline and stress-induced plasma corticosterone concentrations from a subset of individuals. We then fitted all individuals with Passive Integrated Transponder tags, released them at their sites of capture, and monitored their feeder use and social interactions at bird feeders equipped with radio-frequency identification technology. We found that individuals with higher baseline corticosterone concentrations exhibited more exploratory behaviors when introduced to a novel environment. We also found that more exploratory individuals had higher social network connectivity in the wild, though this result was sex-specific and the relationship was stronger for female than for male house finches. Individuals that were quick to begin exploring had higher network connectivity than slow-exploring individuals. Finally, we found that exploratory behaviors were not related to foraging behaviors at bird feeders, which we have previously shown to be predictive of acquiring a common bacterial pathogen, *Mycoplasma gallisepticum*, that causes
annual epidemics in house finches. While we found no evidence that exploratory behavior in house finches should predict the risk of acquiring *M. gallisepticum*, our results suggest that individual differences in exploratory behavior could have important implications for house finch ecology from the individual to the group or population level.

**Keywords:** animal personality, corticosterone, exploratory behavior, social network, house finch (*Haemorhous mexicanus*)

**Introduction**

Animal personalities, or consistent inter-individual differences in behavior (Réale et al., 2010; Sih et al., 2004), influence the ways in which an individual interacts with conspecifics, other species, and the environment (e.g. Aplin et al., 2013; Boyer et al., 2010; Favati et al., 2014; Pruitt and Modlmeier, 2015). Variation in such interactions can have important implications ranging from individual-level responses (e.g. physiology), to group- and population-level dynamics (e.g. transmission of information or disease), to community composition. One of the most commonly studied behavioral heterogeneities in the context of animal personalities is response to novelty, wherein individuals are often placed on a behavioral spectrum that ranges from “bold” (displaying exploratory behaviors) to “shy” (displaying neophobic behaviors) based on their responses to novel environments, objects, and/or conspecifics (e.g. Dingemanse et al., 2007; Drent et al., 2002; Sih et al., 2004; Verbeek and Drent, 1994). This central axis of personality has the potential to serve as a framework for integrating consistent differences in behavior with individual physiology, interactions with conspecifics (e.g. social network position; Aplin et al., 2013; L. S. C. McCowan and Griffith, 2015; Snijders et al., 2014), and interactions
with the environment (e.g. Boon et al., 2008; Boyer et al., 2010). While previous studies have explored different aspects of this framework, none have linked physiology, exploratory behaviors, and behavioral correlates (e.g. sociality), thus limiting our ability to understand whether these traits can interact to influence individual behavior, and ultimately population processes.

Numerous recent studies have suggested that exploratory behavior is linked to components of individual physiology, and much of the work to date has focused on stress physiology. While links between stress physiology and boldness behaviors have been found in several studies, the direction of the relationships between stress hormones (glucocorticoids) and boldness behaviors is not consistent across taxa. For many species of mammals such as mice, rats, and pigs, shy individuals showed higher stress-induced glucocorticoid concentrations than bold individuals (reviewed in Carere et al., 2010). However, for birds, the relationship between personality and concentrations of the glucocorticoid corticosterone (CORT) is less clear. Higher stress-induced CORT concentrations have been correlated with slow exploration tendencies in great tits (*Parus major*) (Baugh et al., 2013, 2012; Carere et al., 2010), house sparrows (*Passer domesticus*) (Lendvai et al., 2011), and dark-eyed juncos (*Junco hyemalis*) (Atwell et al., 2012). However, captive zebra finches (*Taeniopygia guttata*) with higher stress-induced circulating CORT concentrations exhibited more exploratory behaviors than zebra finches with lower circulating CORT concentrations (Martins et al., 2007). Thus, broad generalizable patterns between exploration behavior and stress physiology, at least among birds, have not yet emerged.

There is also accumulating evidence that animal personality and components thereof (i.e. exploratory behaviors) are linked to behavioral traits pertaining to sociality. However, studies linking personality to sociality also have yet to yield broad patterns. For some species, such as
grey kangaroos (*Macropus giganteus*) and zebra finches, shy animals tend to associate with larger groups (Best et al., 2015; L. S. McCowan and Griffith, 2015), presumably because there is safety in numbers (Best et al., 2015). In contrast, shy three-spined sticklebacks (*Gasterosteus aculeatus*) associate with fewer individuals (Pike et al., 2008), and less exploratory great tits hold more peripheral (less central) positions within social networks in the wild (Aplin et al., 2013; Snijders et al., 2014), but maintain more stable social relationships (Aplin et al., 2013).

Investigating how differences in exploratory behavior correlate with differences in social network centrality can provide a broader understanding of how behavioral heterogeneity at the individual level can influence the patterns of how groups of conspecifics interact with one another, i.e. the social structure or social network.

Inter-host heterogeneity in behavior, such as exploration tendency, can potentially influence social processes both at the individual and the population level. For example, being more social or more explorative could increase exposure to infectious agents (e.g. Barber et al., 2010; Dizney and Dearing, 2013; Ezenwa et al., 2016; Fairbanks and Hawley, 2012; Johnson and Hoverman, 2014; Vanderwaal et al., 2013). In some cases, the same behaviors that mediate among-individual variation in exposure to pathogens (e.g. social dominance; Fairbanks and Hawley, 2012) are also associated with physiological mechanisms that either augment or decrease susceptibility once exposed (e.g. social dominance; Habig and Archie, 2015). This behaviorally-mediated covariation in exposure and susceptibility among individuals can, in some cases, allow or prevent pathogen invasion in a population (Hawley et al., 2011). To date, several studies have shown links between personality traits, exposure to parasites (e.g. Boyer et al., 2010; Gyuris et al., 2016), susceptibility to infection (Koprivnikar et al., 2012), and disease transmission (e.g. Keiser et al., 2016). Thus, exploration behavior may be an example of a
behavior that can have dual effects on both the likelihood of exposure to pathogens (via behavioral differences) and susceptibility to infection once exposed (via physiological differences), with important population-level consequences for infectious disease dynamics. Understanding how exploratory behaviors link to both inter-individual differences in exposure and physiology within a single study population will shed light on this possibility.

In this study, we explored the relationships between exploratory behavior, stress physiology, and social and foraging behaviors in wild house finches (*Haemorhous mexicanus*). House finches are common songbirds found across North America, and form loose winter flocks during their non-breeding season (Altizer et al., 2004; Thompson, 1960a). House finches also regularly frequent backyard bird feeders, which make them an excellent species for tracking social behaviors and assessing social network metrics at radiofrequency identification (RFID) equipped feeding stations. House finches frequently interact as they forage, and can be regularly observed aggressively displacing one another from feeder ports as they compete for access to food (Hawley et al., 2006; Thompson, 1960b). Furthermore, the use of bird feeders by house finches has been linked to the risk of transmission of a naturally occurring bacterial pathogen, *Mycoplasma gallisepticum* (Adelman et al., 2015; Dhondt et al., 2007; Hartup et al., 1998), that has caused annual outbreaks in eastern North American house finch populations since the mid-1990s (Fischer et al., 1997; Altizer et al. 2004). Individual house finches that spend more time on feeders are both more likely to acquire and spread *M. gallisepticum* (hereafter Mg) (Adelman et al., 2015).

We first examine whether exploratory behavior is linked to stress physiology in the house finch. To do this, we caught wild house finches during the non-breeding season and assayed their response to a novel environment before banding, PIT-tagging, and releasing them. We assessed
baseline and stress-induced circulating CORT concentrations for a subset of these birds upon capture. In line with the patterns found in many species of songbirds, we predicted that more exploratory house finches would have lower CORT concentrations than less exploratory conspecifics.

In addition to exploring the hormonal basis of exploratory behavior, we then test whether exploratory behavior in wild house finches is linked to inter-individual variation in three types of behaviors in the wild: foraging behaviors, aggressive behaviors, and social network metrics. Because exploratory behavior could be linked to movement across the landscape or to risk-taking behaviors (e.g. Quinn et al., 2011; Stuber et al., 2013) such as foraging at feeders in open habitat (Dunn and Tessaglia, 1994), we predicted that more exploratory house finches would visit more unique feeders in our study population and spend more overall time on bird feeders than less exploratory birds. This is important, because the number of feeders individual house finches visit, or the amount of time they spend at feeders in the wild, has been previously suggested as a major factor underpinning Mg transmission among house finches. Additionally, in line with patterns observed in several taxa (e.g. Huntingford, 1976; Sih et al., 2004; Verbeek et al., 1996), we predicted that more exploratory birds would engage in more aggressive interactions than less exploratory birds. Lastly, as has been shown in great tits (Aplin et al., 2013), we predicted that more exploratory birds would exhibit higher sociality and thus hold a more central position within social networks in the wild than less exploratory birds. There is increasing evidence that network centrality can influence disease transmission across a range of taxa (e.g. Bull et al., 2012; Drewe, 2010; Fenner et al., 2011; Keiser et al., 2016; Vanderwaal et al., 2013)
Methods

Field Captures

Wild house finches (n=88) were captured between November 2012 and March 2013 on and around the Virginia Tech campus in Blacksburg, VA. We trapped twice per week throughout this time period, rotating through six field sites so that each site was trapped at once per week. The six sites were located within close enough proximity that finches could feasibly visit all of the locations. One tube-shaped bird feeder was installed at each of our field sites for the duration of our field season. All birds were caught using wire traps suspended around bird feeders or mist nets placed in close proximity of bird feeders.

Quantification of circulating corticosterone concentrations

We quantified the concentrations of circulating CORT in a subset of 20 birds captured during our field season for which we were able to obtain both a baseline and stress-induced blood sample. Because not all of the 20 birds that we obtained CORT samples for remained in the social network long enough (≥7 days) to be included in the field study (see below), there is very small overlap (n=8) in the individuals for which we had CORT, exploratory behavior, and social network data. Thus, the two overarching research questions (how CORT relates to exploratory behavior, and how exploratory behavior relates to social and feeding behaviors) were analyzed separately using largely distinct individuals.

For birds bled for CORT, initial blood samples were taken within three minutes of entry into the trap or net to quantify baseline CORT concentrations, and a subsequent blood sample was taken at 30 minutes post-capture to quantify stress-induced CORT concentrations (Romero and Reed, 2005). Blood was collected by puncturing the brachial vein with a sterile 26-gauge needle, and then collecting the pooled blood (~50-100 microliters) using a heparinized capillary
tube. Samples were stored on ice for up to six hours until centrifugation and plasma extraction. Plasma was stored at -20°C. CORT concentrations were quantified using direct radioimmunoassay following extraction in re-distilled dichloromethane (Bonier et al., 2011). We used the corticosterone antibody from Esoterix Endocrinology (catalogue number B3-163) and labeled corticosterone from New England Nuclear Research Products (catalogue number NET-399). Samples were corrected individually for extraction efficiency, and average extraction efficiency was 72.0%. Within-assay variation among four known-concentration standard samples was 9.65%, and the detection limit was ~1.4 ng/ml.

Temporary Housing

Immediately following capture, all birds were fitted with a United States Geological Survey aluminum band stamped with a unique ID number and several morphometric measurements were taken (mass, wing length, tarsus size, and pectoral muscle index—a metric of the robustness of the pectoralis major, where a larger number indicates a more robust muscle). A blood sample was taken from all individuals before being temporarily housed, but only the individuals for which we were able to obtain a blood sample within three minutes of capture were included in the CORT study. Following processing, all birds were temporarily housed within an indoor animal care facility on the campus of Virginia Tech for approximately 24 hours. Birds were held individually in visually isolated small cages (46 x 46 x 76cm) and provided ad libitum food and water. All birds were housed in the same indoor room at a constant light cycle (12L:12D) and temperature (22-24°C). Individuals captured with clinical signs of mycoplasmal conjunctivitis (swelling, redness, or exudate in either eye) were not held overnight to avoid contaminating the novel environment arena. All such individuals were released at the site of capture after banding and processing (see below) and were not included in this study.
Response to Novel Environment

After temporary overnight housing (as per Aplin et al., 2013), we placed each bird individually into a small wooden refuge (25.4 x 25.4 x 25.4cm), placed the refuge inside a novel environment arena, and left the room. After approximately five minutes within the refuge, a sliding door on the refuge was remotely opened using a pulley system, allowing the bird access to the closed observation arena (2 x 2.3 x 2.6m). The observation arena contained five identical wooden trees, each with four branches of varying heights (as per Verbeek et al., 1994). A video camera located just outside of the arena recorded the behavioral response of each bird for 15 minutes to quantify exploratory behavior via response to this novel environment (Verbeek et al., 1994).

To quantify behavioral response to a novel environment, the following non-independent behaviors were quantified and incorporated into a principal components analysis (as per Dingemanse et al., 2002): latency to exit the refuge, latency to perch on first tree, number of trees visited, number of inter-tree flights, total number of flights, number of inter-perch hops, number of unique perch visits, total number of perch visits, and number of refuge returns. Principal components analysis of behavioral response to a novel environment was performed using R (R Development Core Team, 2014, see analysis below).

Banding, PIT Tagging, and Release

After completing the novel environment assay, all birds were given a unique combination of color bands, and fitted with a passive integrative transponder (PIT) tag. Each 0.1-gram PIT tag was fastened to the color bands on the right leg using colored electrical tape (as per Bonter et al., 2013). Birds then had their conjunctiva swabbed for a separate study (Adelman et al., 2015) and were released at their site of capture.
Monitoring Behavior of PIT-Tagged Birds

At each of our field sites, we placed bird feeders fitted with Radio Frequency Identification (RFID) reading technology to monitor the social and foraging behavior of PIT-tagged birds at our feeders (as per Aplin et al., 2013; Bridge and Bonter, 2011). Each tube-shaped feeder had two ports, each with an RFID antenna affixed below the perch. These antennas, which functioned independently of one another, logged the presence of PIT-tagged birds at a resolution of one data point per second. This allowed us to collect data on the social interactions between PIT-tagged individuals, as well as feeding behaviors at RFID-equipped feeders. We estimate that we PIT-tagged approximately 49% of the population (Adelman et al., 2015).

Extracting Behavioral Metrics Using Radiofrequency Identification Data

We extracted three categories of behavioral metrics: social network metrics, aggressive interactions, and foraging behavior, all of which have been shown to predict disease transmission in house finches and/or other taxa, and are measurable using RFID technology. To test whether exploratory tendencies predicted a bird’s relative centrality within a social network, we used the methods described in Adelman et al. (2015) to construct a foraging network from our RFID data based on patterns of co-occurrence in foraging flocks (Psorakis et al., 2015, 2012) at our RFID-equipped feeders using the R package asnipe (Farine, 2013; Farine and Whitehead, 2015). Edges in our network were defined using the simple ratio index, or the probability of observing two individuals together given that one was observed. We used these networks to determine each bird’s weighted degree and eigenvector centrality (metrics used to describe an individual’s position within a social network) using the sna package for R (Butts 2014).
To measure social dominance, aggressive inter-individual interactions at the feeder were inferred by quantifying each time two individuals were logged at the same feeder port within a two-second window of one another, suggesting that an aggressive displacement event occurred (Adelman et al., 2015). For each of these interactions, a bird was considered the winner if it displaced another individual; a bird was considered the loser if it was displaced by another individual. From these data, we calculated social dominance as each bird’s Elo score (Neumann et al., 2011), computed in the AniDom package for R (Sanchez-Tojar, A., Schroeder, J., Farine, D.R. (in prep)). Finally, we used RFID data to extract two metrics of foraging behaviors in the wild. First, we quantified the average amount of time individuals spent on the feeders per day, because this behavior has been shown to predict the spread of MG in house finches (Adelman et al., 2015). Second, we examined the average number of different bird feeders an individual visited per day, because this could be a relevant metric of spatial exploration in the wild.

Statistical Analyses

Exploratory Behavior

We used principal component analysis in R (R Development Core Team, 2014) to describe variation in the many non-independent behaviors we measured in response to the novel environment assay. All birds included in the CORT and Exploratory Behavior study and/or the Exploratory Behavior and Field Behaviors studies were included in this analysis (n=64). The first two principal components together explained 71.5% of the observed variation in behavioral response to a novel environment. Principal component 1 (PC1; Exploration), which explained 52.8% of variation, was essentially a metric of the amount of exploration, and was positively correlated with the number of unique perches visited, the number of trees visited, number of
flights, total number of perch visits, and number of inter-perch hops (Table 1). Principal component 2 (PC2; Exploration Latency), which explained 18.7% of variation, was a metric of latency to explore and proclivity for sitting on the ground. PC2 was positively correlated with latency to exit the refuge and latency to perch on the first tree, as well number of visits to the ground. PC2 was negatively correlated with the number of times a bird perched on an object other than a tree or perch (mesh boundaries of observation arena, etc.).

**Corticosterone and Exploratory Behavior**

We used general linear models in R (R Development Core Team, 2014) to assess relationships between stress physiology and response to a novel environment. The dependent variable was either Exploration (PC1), or Exploration Latency (PC2). Our predictor variables were baseline CORT concentrations and stress-induced CORT concentrations, and we included several potential relevant covariates in each initial model (sex, mass, and pectoral muscle index). Initial models included all pairwise interactions, but interactions that were not significant at an alpha level of 0.05 were removed from the final model. Mass did not significantly predict either PC1 or PC2, and thus, was not included in the final models.

**Exploratory Behavior and Field Behaviors**

We tested whether exploratory behavior in a novel environment was related to two metrics of social network position. Because of issues with non-independence in network data, standard statistical practices are not suited for analyses of animal social networks (Croft et al., 2011; Farine and Whitehead, 2015). We therefore used randomization tests to assess relationships among exploratory behaviors and social network metrics in the field. First, using our observed social networks, we fit separate general linear models in R (R Development Core Team, 2014) for weighted degree and eigenvector centrality. Although all observed birds
(including 62 PIT-tagged birds for which we did not have novel environment assay data) were included in developing the network, only birds with \( \geq 7 \) days of RFID data (n=52) were included in these analyses to exclude transient individuals for which we lacked repeated observations (Adelman et al., 2015). Our initial predictor variables were *Exploration* (PC1), *Exploration Latency* (PC2), sex, and the two-way interactions with sex. To determine whether each of these parameters significantly predicted the dependent variable, we conducted 25,000 permutations of our network in the asnipe package in R (Farine and Whitehead, 2015; Farine, 2013), generating an increasingly random set of networks following the permutation procedure described by Bejder et al. (1998) and restricting swaps to happen within feeder within day. We then ran the same general linear models as above on each of these networks, generating a null distribution for each parameter estimate. If a given variable’s parameter estimate from our observed network fell outside of the 95% range of the estimates from the random networks, the effect of that variable was considered significant. P values calculated using randomizations are denoted \( P_{\text{rand}} \).

Interactions not meeting the significance criterion were removed from the models by backwards elimination.

To determine whether exploratory behaviors were related to other behaviors in the field, we performed general linear models in R (R Development Core Team, 2014). As above, only birds with \( \geq 7 \) days of RFID data (n=52) were included in these analyses. The dependent variables examined were: aggressive interactions per day and relative social dominance (metrics of aggressive behavior), and time spent on feeders per day and the number of unique feeders visited per day (metrics of foraging behavior). Our predictors were Exploration (PC1) and Exploration Latency (PC2), and sex was included as a fixed effect in all models. Our aggression analysis also included each individual’s average number of feeding bouts per day as an
additional covariate. Initial models included all pairwise interactions, but interactions that were not significant at an alpha level of 0.05 were removed from the final model.

Permits

Both studies were conducted under the following permits: Virginia Tech Institutional Animal Care and Use Committee, Virginia Department of Game and Inland Fisheries (038781 and 044569), United States Fish and Wildlife Service (MB158404-1), and United States Geological Survey Bird Banding Lab (23513).

Results

Circulating corticosterone concentrations and exploratory behavior

We found that baseline CORT concentrations at capture, as well as pectoral muscle index, significantly predicted the extent of exploration in the novel environment assay. Baseline CORT concentrations ranged from 0.03-8.79 ng/ml (mean=0.30ng/ml). In contrast to our prediction, house finches that had higher baseline CORT concentrations exhibited more exploratory behaviors (aka had higher Exploration values (higher PC1) in our novel environment assay; baseline CORT: \( \beta=0.78\pm0.24, F_{1,16}=7.56, P=0.015 \) (Figure 1). Additionally, we found that finches that had a higher pectoral muscle index exhibited more exploratory behaviors (higher PC1) than birds with lower pectoral muscle index (pectoral muscle index: \( \beta=3.09\pm1.31, F_{1,16}=5.56, P=0.032 \)). Stress-induced CORT concentrations ranged from 12.56-75.97 ng/ml (mean=38.7ng/ml). Stress-induced CORT concentrations (sample taken 30 minutes post-capture) did not significantly predict individual responses to a novel environment (stress-induced CORT: \( \beta=-0.023\pm0.025, F_{1,16}=0.059, P=0.81 \)). For the subset of birds included in our CORT study, sex
did not significantly predict Exploration in our novel environment assay (sex: $\beta=-0.19\pm1.00$, $F_{1,16}=0.035$, $P=0.85$).

Exploration Latency (PC2) was not significantly predicted by either baseline or stress-induced CORT concentrations (baseline CORT: $\beta=-0.003\pm0.17$, $F_{1,16}=0.00$, $P=0.98$; stress-induced CORT: $\beta=-0.034\pm0.017$, $F_{1,16}=3.89$, $P=0.067$). In contrast to results for Exploration, pectoral muscle index did not significantly predict Exploration Latency ($\beta=-1.24\pm0.91$, $F_{1,16}=1.87$, $P=0.19$). Sex predicted Exploration Latency in the subset of birds assayed for CORT concentrations ($\beta=1.49\pm0.69$, $F_{1,16}=4.65$, $P=0.048$), with males showing lower exploration latency than females.

**Exploratory behavior and feeding behaviors in the wild**

Overall, response to a novel environment did not predict foraging behaviors in free-living house finches. Exploration (PC1) was not associated with the average time (in minutes) individuals spent on bird feeders per day ($\beta=-0.029\pm0.55$, $F_{1,51}=0.003$, $P=0.96$), nor was Exploration Latency (PC2) ($\beta=-1.32\pm0.91$, $F_{1,51}=2.12$, $P=0.15$). Sex also was not a significant predictor of average time spent on feeders per day ($\beta=-2.54\pm2.44$, $F_{1,51}=1.08$, $P=0.30$). Additionally, Exploration (PC1), Exploration Latency (PC2), and sex were not significant predictors of the average number of feeders that house finches visited per day (PC1: $\beta=-0.009\pm0.005$, $F_{1,51}=3.21$, $P=0.080$; PC2: $\beta=-0.003\pm0.009$, $F_{1,51}=0.126$, $P=0.72$; Sex: $\beta=0.00\pm0.023$, $F_{1,51}=0.00$, $P=0.99$).

**Exploratory behavior and aggression in the wild**

The number of feeder visits an individual made per day was a strong predictor for the average number of aggressive interactions an individual experienced (as an initiator or a
receiver) per day (β=0.11±0.011, F_{1,50}=104.05, P<0.001). However, we found no relationship between *Exploration* (PC1), *Exploration Latency* (PC2), or sex and the number of aggressive interactions per day at our RFID equipped feeders (PC1: β=0.075±0.048, F_{1,50}=2.48, P=0.122; PC2: β=-0.035±0.082, F_{1,50}=0.18, P=0.68; Sex: β=-0.063±0.21, F_{1,50}=0.089, P=0.77).

Furthermore, *Exploration* (PC1) and *Exploration Latency* (PC2) were not positively correlated with Elo score, a measure of relative social dominance at bird feeders (PC1: β=2.13±1.86, F_{1,50}=1.31, P=0.26; PC2: β=-1.25±3.23, F_{1,50}=0.15, P=0.70). While not significant, females tended to have lower Elo scores, and thus be less dominant than males (β=-16.37±8.33, F_{1,50}=3.86, P=0.055).

*Exploratory behavior and social network metrics in the wild*

Our permutation analyses detected significant effects of both *Exploration* and *Exploration Latency* on weighted social network degree in the wild. The effects of *Exploration* (PC1) were sex-specific (sex(F) x PC1: β=0.018±0.049, P_{rand}=0.028). The positive relationship between *Exploration* (PC1) and weighted degree, indicating that more exploratory house finches interacted with more unique conspecifics than less exploratory house finches, was stronger for females (β=0.062) than it was for males (β=0.044) (Figure 2). The main effect of sex was also significant in our model, with female house finches having a higher weighted degree (β=0.069±0.086, P_{rand}=0.0003). A significant relationship between *Exploration Latency* (PC2) and weighted degree was present for both sexes: consistent with our prediction, birds that exhibited higher exploration latencies (higher PC2 = less exploratory) had lower weighted degrees (β=-0.04±0.034, P_{rand}=0.004).
Neither *Exploration* (PC1) nor *Exploration Latency* (PC2) significantly predicted eigenvector centrality in the wild (PC1: $\beta=0.10\pm0.13$, $P_{\text{rand}}=0.28$; PC2: $\beta=0.23\pm0.22$, $P_{\text{rand}}=0.37$). Furthermore, sex did not significantly predict eigenvector centrality ($\beta=-0.47\pm0.56$, $P_{\text{rand}}=0.24$). However, our certainty about these results are low given that broader network metrics, such as eigenvector centrality, are more meaningful in larger networks.

**Discussion**

Our results suggest that exploratory behavior in a novel environment is linked to both CORT and sociality in free-living house finches. We found that baseline CORT concentrations were correlated with *Exploration* (PC1) in a novel environment, linking physiology to exploratory behaviors in this system. However, stress-induced CORT concentrations were not correlated with *Exploration* (PC1) or *Exploration Latency* (PC2). Additionally, we found links between exploratory behaviors and sociality as both *Exploration* (PC1) predicted weighted degree in the wild, and *Exploration Latency* (PC2) predicted weighted degree in the wild. However, we were unable to show a direct link between circulating CORT concentrations and social interactions in the wild due to the small number of birds (n=8) for which we had sufficient data on both CORT concentrations and social behaviors in the field. Nevertheless, our data suggest a possible link between individual physiology and the ways in which individuals interact with one another at the group level, mediated through inter-individual behavioral variation in free-living house finches. Thus, our study contributes to a broader understanding of the ecological consequences of individual variation in physiology and behavior.

We found that baseline circulating CORT concentrations were correlated with the extent of exploration of a novel environment in house finches. However, in contrast with our
predictions and previous work done with great tits (Baugh et al., 2013; Stöwe et al., 2010), more exploratory birds (as measured by PC1) had higher baseline CORT concentrations than less exploratory birds at the time of capture. Our results were more similar to the pattern detected in a study of zebra finches, where bolder individuals have higher concentrations of circulating CORT than their shy counterparts (Martins et al., 2007). However, this pattern was found only after the zebra finches were exposed to a stressor, whereas we found no significant differences in stress-induced CORT concentrations between “bold” and “shy” individuals. One possible explanation for this discrepancy is that the zebra finches from the Martins et al. (2007) study were selected for high and low CORT reactivity lines, thus increasing their likelihood of detecting varying levels of CORT reactivity in response to a stressor. Further study is needed to determine why the patterns we detected between CORT concentrations and exploratory behaviors in free-living house finches differ from those of great tits, which have otherwise similar social systems during the non-breeding system.

Overall, our results suggest that exploratory behavior and stress physiology are linked in house finches. However, because our study was observational, we are not able to establish a causal relationship between CORT concentrations and exploratory behavior. It is possible that birds with higher baseline CORT are able to better mobilize their energy stores, allowing them to engage in more exploratory behaviors. It is also possible that higher energy mobilization could lead to an increased requirement for food intake, necessitating more exploratory behaviors in search of potential food sources. Although our data cannot distinguish between these and other non-mutually exclusive possibilities, we found that birds with higher pectoral muscle index exhibited more exploratory behaviors during the novel environment assay. This suggests that the relationship between CORT and exploratory behavior may be mediated in part by differences in
body condition. We did not find any evidence that CORT was directly associated with pectoral muscle index in our study, but our sample sizes may have been insufficient to detect such relationships. Additionally, our small sample size may have limited our power to detect a relationship between stress-induced CORT and exploratory behavior. Experimental studies are needed to confirm any causation underlying the detected links, and thus to better understand the physiological mechanisms driving variation in individual exploration behavior.

We also found that exploratory behaviors were predictive of house finch social behavior in the wild. House finches that exhibited more exploratory behaviors in response to a novel environment (higher PC1) had a higher weighted social network degree in the wild, and this relationship was stronger for female house finches. The positive relationship between exploration and weighted degree is consistent with our prediction and with past studies on great tits (Aplin et al., 2013; Snijders et al., 2014). However, further work is needed to determine why exploration was not as strongly linked to network degree in male house finches. Past work suggests that sexual selection could be a driver of network metrics for male house finches, but this study did not include female house finches (Oh and Badyaev, 2010). For our other metric of exploratory behavior, Exploration Latency, we found that house finches that were slower to explore the novel environment had significantly lower weighted degree, and this pattern was not influenced by sex. This suggests that, as has been shown in great tits (Aplin et al., 2013; Snijders et al., 2014), birds that are very slow to begin exploring (or never leave the refuge) during the novel environment assay interact significantly less often with unique conspecifics in the wild. Overall, our results indicate that both Exploration and Exploration Latency are linked with sociality in free-living house finches, and this link may have important consequences for population-level processes.
Surprisingly, neither relative social dominance (measured using Elo scores) or the rate of aggressive interactions was significantly tied to exploration activity in our study. This unexpected result contrasts with behavioral patterns found in both great tits (e.g. Dingemanse and De Goede, 2004; Verbeek et al., 1996) and zebra finches (David et al., 2011), wherein more exploratory birds initiate and win more agonistic interactions than less exploratory birds. The discrepancy between our findings and those in other songbird species may be a result of the limitations inherent in using RFID-equipped feeders to measure social dominance and aggressive interactions. First, the only social dominance or agonistic interactions that can be inferred from RFID data are those in which a bird sitting on the feeder is successfully displaced by a more dominant bird, which we define as a unique bird being detected within two seconds of the prior bird’s departure. Our definition of an aggressive interaction thus excludes instances where, for example, very shy or subordinate birds readily departed the feeder at any sign of approach made by a dominant conspecific. Second, our RFID data cannot identify instances where a bird sitting on the feeder is challenged by another, but asserts its dominance and does not abandon its position. Thus, our approach likely excludes interactions between dyads that include a very dominant or very subordinate bird. This combination of factors could truncate the variability of social interactions that we were able to observe via RFID at both extremes of the social status spectrum. However, these limitations are likely outweighed by our ability to record many agonistic interactions over long periods of time by using automated technology.

Finally, we predicted that more exploratory birds, which readily moved around and explored a novel environment, would move across the landscape more readily and visit more unique bird feeders per day. However, our data did not show a significant link between exploratory behaviors in a novel environment and the number of feeders used in the wild. It is
possible that our feeders were placed far enough apart that we were not able to detect subtle
variation in feeder use across a landscape, as the median number of feeders used by a bird in our
study was two. Additionally, the use of bird feeders across a landscape could be determined at
the group level rather than at the individual level, thus obscuring differences in exploratory
behaviors among individuals within a group (flock). Finally, exploratory behavior was also not a
significant predictor of the amount of time that house finches spent on feeders, a behavior that
has been shown to influence disease dynamics for house finches in the past (Adelman et al.
2015). Together, these results suggest that exploratory behavior in free-living house finches does
not predict inter-individual variation in interactions with bird feeders.

Conclusions

This study highlights the importance of examining connections between individual
physiology and behavior for understanding how these influence group-level dynamics, and in
turn could potentiate population processes, such as disease transmission. Together, our results
suggest that exploratory behavior may be linked to both behavioral and physiological traits
important for disease transmission. However, we did not find a link between exploratory
behavior and the feeding behaviors that have been previously shown to influence Mg dynamics
in house finches. While our results suggest that exploratory behavior may not shape the
likelihood of a house finch acquiring Mg, the links between stress physiology, exploratory
behavior, and sociality could work in concert to influence the dynamics of diseases transmitted
primarily through social interactions.

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**References**


doi:10.1093/beheco/arh115


doi:10.1016/j.anbehav.2013.08.003


doi:10.1098/rspb.2015.3078


Farine, D.R., 2013. Animal social network inference and permutations for ecologists in R using


Hawley, D.M., Etienne, R.S., Ezenwa, V.O., Jolles, A.E., 2011. Does animal behavior underlie


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,
Sociobiol. 69, 857–866. doi:10.1007/s00265-015-1906-0


in.

approaches to the study of personality. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 365, 3937–
3946. doi:10.1098/rstb.2010.0222

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: is under
doi:10.1016/j.cbpb.2004.11.004


networking in territorial great tits: Slow explorers have the least central social network


Table 1.
Loading values for the two principal components used as measures of *Exploration* (PC1) and *Exploration Latency* (PC2).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>PC1 ('Exploration')</th>
<th>PC2 ('Exploration Latency')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency to Exit Refuge (s)</td>
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<td>0.73*</td>
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<tr>
<td>Latency to First Tree</td>
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<td>0.67*</td>
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<tr>
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<td>-0.03</td>
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<tr>
<td>Total Number of Flights</td>
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<td>Inter-Perch Hops</td>
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<tr>
<td>Number of Unique Perches Visited</td>
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<td>Total Number of Perch Visits</td>
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<tr>
<td>Number of Visits to Non-Perch</td>
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<tr>
<td>Number of Ground Visits</td>
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<td>0.19</td>
</tr>
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</table>

*Significant loading values (values ≥0.4)
Figure 1.

House finches with higher circulating baseline corticosterone concentrations at capture exhibited higher *Exploration* (PC1) in a novel environment assay, when accounting for pectoral mass index.
Figure 2.

The extent of exploratory behaviors (Exploration (PC1)) in a novel environment assay had sex-specific but positive effects on weighted degree in free-living house finches.
Chapter III: Feeder use predicts both acquisition and transmission of a contagious pathogen in a North American songbird

Co-authors: James S. Adelman, Damien R. Farine, Dana M. Hawley


Abstract

Individual heterogeneity can influence the dynamics of infectious diseases in wildlife and humans alike. Thus, recent work has sought to identify behavioural characteristics that contribute disproportionately to individual variation in pathogen acquisition (super-receiving) or transmission (super-spreading). However, it remains unknown whether the same behaviours enhance both acquisition and transmission, a scenario likely to result in explosive epidemics. Here we examined this possibility in an ecologically relevant host-pathogen system: house finches and their bacterial pathogen, Mycoplasma gallisepticum, which causes severe conjunctivitis. We examined behaviours likely to influence disease acquisition (feeder use, aggression, social network affiliations) in an observational field study, finding that the time an individual spends on bird feeders best predicted the risk of conjunctivitis. To test whether this behaviour also influences the likelihood of transmitting M. gallisepticum, we experimentally inoculated individuals based on feeding behaviour and tracked epidemics within captive flocks. As predicted, transmission was fastest when birds that spent the most time on feeders initiated
the epidemic. Our results suggest that the same behaviour underlies both pathogen acquisition and transmission in this system and potentially others. Identifying individuals that exhibit such behaviours is critical for disease management.

**Keywords**: disease transmission, behavioural heterogeneity, house finch (*Haemorhous mexicanus*), *Mycoplasma gallisepticum*, super-spreader, super-receiver

**Introduction**

Heterogeneity in exposure and response to pathogens can strongly influence the duration and intensity of disease epidemics [1]. Although traditional models of disease dynamics frequently treat individuals as homogeneous or randomly variable (*e.g.*, [2]), in reality, a small percentage (20%) of a population is often responsible for the majority (80%) of pathogen transmission (*e.g.*, [3]). Such disproportionate contributions to acquiring or spreading pathogens have been termed “super-receiving” [4], and “super-spreading” [5], respectively. Given the importance of individual heterogeneity to infectious disease dynamics [1], there is growing interest in identifying which individuals will be super-receivers or super-spreaders in both human and wildlife populations (*e.g.*, [6-9]).

Host behaviour is a strong driver of heterogeneity in pathogen acquisition or spread for numerous systems [10-14]. Among wildlife, variation in territoriality (*e.g.*, [10]), social status (*e.g.*, [7]), foraging [15], social network connectivity (*e.g.*, [14, 16]), grooming [17], and aggression [4, 17] have all been shown to underlie individual variation in exposure to infectious agents (super-receiving). Among humans, variation in behavioural traits such as hand hygiene
and social contacts [5] among healthcare workers can increase super-spreading in severe acute respiratory syndrome. Similarly, high connectedness in human sexual networks can increase the chance of acquiring and spreading sexually-transmitted disease [19]. However, behavioural predictors of super-spreading in wildlife remain largely unknown because of the lack of detailed contact tracing.

Although behaviour can be important for predicting individual variation in pathogen acquisition and spread in some systems, it remains unknown whether the same behaviours make an individual both more likely to be a super-receiver and more likely to be a super-spreader. In the case of Tasmanian Devil Facial Tumour Disease, biting conspecifics is a strong predictor for acquiring disease, while being bitten is not [4]. Thus, while aggressive Tasmanian Devils are likely super-receivers, they are unlikely to be super-spreaders. In other systems, however, the same behaviours can influence both pathogen acquisition and transmission. For example, host defensive behaviours against arthropods can influence both exposure to vector-borne pathogens and the capacity to spread these pathogens [20, 21]. In such systems, where the same individuals are both super-receivers and super-spreaders, behavioural covariation in exposure and infectiousness could have significant epidemiological consequences [22]. Here, we examine this issue through field and experimental studies in an emerging wildlife host-pathogen system: house finches (Haemorhous mexicanus) and their bacterial pathogen Mycoplasma gallisepticum (MG).

Since the mid 1990’s, the house finch, a common North American songbird, has been host to an emerging clade of MG [23], historically a poultry pathogen. In house finches, MG causes severe conjunctivitis [24] and has been associated with significant population declines [25]. Since MG’s
emergence in finches, annual winter epidemics have been observed throughout the United States (US) [26]. This pattern is strongly associated with the social behaviour of non-breeding house finches: forming loose flocks and congregating around bird feeders [26, 27].

The house finch-MG system provides a powerful context for testing how variation in behaviour predicts the risk of acquiring and spreading infection. House finches are highly social during the relevant period of MG transmission. Direct interactions between individuals are likely critical for transmission because MG, like all Mollicute bacteria, lacks a cell wall precluding it from surviving for long outside of its host [28]. Furthermore, indirect transmission via fomites (i.e., objects capable of harbouring infectious organisms) is also possible in this system [29]. Moreover, controlled experiments can be readily conducted [30], enabling us to quantify how different individual-level behaviours relate to pathogen transmission.

Here we examine three classes of behaviours potentially associated with super-receiving and super-spreading in this system: social network position, aggressive interactions at feeders, and foraging behaviours. First, given that gregarious individuals likely come into contact with more conspecifics, we predicted that house finches most central in the social network and those associating in larger flocks would more likely act as super-receivers, via direct or indirect transmission. Second, because agonistic interactions are the primary source of direct contacts during the non-breeding season (when MG prevalence peaks) [27], we predicted that birds with more frequent or diverse displacements at feeders would be more likely to acquire disease if direct transmission is important to transmission. Third, given that feeders can serve as fomites of MG [29], we predicted that finches using feeders more often would be more likely to show
conjunctivitis if indirect transmission is most important in this system. We tested these predictions in free-living house finches using radio-frequency identification (RFID) and repeated captures over the course of five months. After determining the behavioural traits associated with super-receiving in the field, we initiated replicated experimental epidemics in captive flocks by inoculating “index” birds exhibiting different behaviour to assess whether the behaviours associated with super-receiving are also associated with super-spreading.

Methods

I. Field Study

Initial Captures and Temporary Housing

From October 2012 – February 2013, we captured house finches across six sites on and near Virginia Tech’s campus using baited traps and mist nets. Trapping occurred twice per week (only once per week at each site). Sites were close enough that birds could use all six (maximum distance between sites = 2.3km), though most individuals used fewer (median = 2, range = 1-5). We captured and marked 180 individuals, of which 117 were detected via RFID at least once and 35 were physically recaptured (median times recaptured = 1, range = 1-3). We estimated the local population size to be 364 (see Supplemental Material (SM)), suggesting that we marked roughly 49% of the population, with 32% of the population (n=117 detected via RFID) contributing to our social network (see below). Recent work suggests that marking 30% of a population of this size likely yields accurate metrics of an individual’s relative connectedness within a social network [31].
Birds were housed overnight for a separate study (see SM), fitted with plastic leg-bands, a US Fish and Wildlife Service aluminium band, and a unique passive integrated transponder (PIT) tag, which was secured to plastic bands using electrical tape [32]. Similar tags do not affect fitness in other small passerines [33]. No detached PIT tags were detected during the study. Birds were released at their capture site within 22-30 hours.

Assessing Infection
All birds were scored for clinical signs of Mycoplasmal conjunctivitis on an “eye score” scale (0 to 3 per eye) as described elsewhere and in SM [30]. Eye score provides a non-invasive, accurate proxy for infection: we tested a subset of wild-caught animals for the presence of MG using qPCR and found strong agreement with eye score (SM, Fig. S1).

Monitoring PIT-tagged Birds
At all sites we installed a bird feeder equipped with an RFID system [34] to log visits by PIT-tagged individuals. Feeders were tube-type, with two feeding ports. Below each port, we placed an RFID antenna, connected to a reader that logged one data-point per second. Batteries were changed regularly to ensure uninterrupted logging.

Extracting Behavioural Metrics from RFID Data
We quantified three classes of behaviours (social networks, aggressive interactions, and foraging behaviours) using our RFID data. Although house finches also have social interactions at non-feeder locations, house finches in the eastern US depend heavily upon feeders [35-37]. These sites are the primary source of contact during the non-breeding season, suggesting that
behavioural interactions at feeders are particularly relevant to pathogen transmission. Moreover, numerous studies have shown that flock membership inferred from feeders predicts the spread of information through populations and captures broader social interactions relevant to fitness (e.g., [38, 39]). Although single instances of co-occurrence contain little information for constructing social networks, analysing aggregate patterns of co-occurrence across many instances (in our case thousands of records) is key to generating robust networks [40, 41].

Social Networks. To assess whether social networks predicted the risk of conjunctivitis, we first applied a machine learning algorithm (Gaussian Mixture Model, [42]) to identify co-feeding events. This algorithm identifies clusters of detections on feeders, by evaluating the non-uniform (or ‘bursty’) data-stream, circumventing the need to use arbitrary time thresholds when defining associations. We next used the R package asnipe [43] to generate a network based on patterns of co-occurrence by individuals in the same feeding events. We then defined associations between birds (network edges) using the Simple Ratio Index (SRI). This represents the probability of observing two individuals together given that at least one was observed (e.g., 0 for dyads never observed together, 1 for dyads always observed together).

Using these foraging networks, we estimated each bird’s position within the social network using weighted degree and eigenvector centrality in the sna package for R [44]. These metrics reflect a bird’s local connectedness and network-wide connectedness, respectively, and are predictive of information acquisition [38] and infection in other wildlife systems [6-8]. Because flock size has been associated with higher prevalence of Mycoplasmal conjunctivitis in finches [26], we calculated each bird’s average adjusted group size. Adjusted group size was defined as the
number of marked individuals in each foraging flock divided by the total number of marked
individuals detected that day.

*Aggressive Interactions.* To quantify the propensity for direct contact among conspecifics, we
quantified displacement events at feeders. We defined displacement as the detection of two birds
at the same feeder port within 2s of one another. For each individual, we calculated the average
number of total displacements in which that bird was involved per day, as displacing or being
displaced could equally lead to physical contact. We also calculated the average number of
unique individuals per day with which a focal bird was involved in displacements. Finally, we
used displacements to calculate relative dominance, using the residuals of a regression between
times displaced versus times displacing. A positive relative dominance value means that an
individual displaced others more often than it was displaced.

*Foraging behaviours.* We extracted three metrics of foraging behaviour hypothesized to be
relevant for acquiring MG: 1) the total amount of time individuals were logged at feeders per
day, 2) the number of unique feeders visited per day, and 3) the total number of feeding bouts
individuals made, assuming a 4s or longer gap in detection of the same individual indicated
separate bouts.

*Statistical Analyses: Field Study*

Because we relied on repeated observations of individuals to robustly quantify behaviours
relevant to disease acquisition, and we had a significant proportion of transient individuals in our
population, we limited our final analysis of disease risk to individuals that were detected by
RFID on seven or more days (n=76, 35 of whom were also physically captured multiple times) [45]. However, since transient birds still formed part of the flocks, we included all individuals ever detected by RFID (n=117) when constructing the social network (including identifying groups) and generating metrics on aggressive interactions.

To determine which behaviours are associated with Mycoplasmal conjunctivitis in the wild, we used generalized linear models with binomial error distributions in R [46]. The dependent variable was whether an individual was ever detected as diseased (1) or not (0). Models were compared and parameters averaged using AICc [47] in the MuMIn package for R [48]. Relative importance of each parameter was calculated by summing the AICc weights from all models in which that variable occurred. Independent variables are listed in Table 1. For each bird, rates were calculated as a total from the entire study divided by the number of days on which that bird was detected. The number of times captured was included in every model to control for potential detection bias whereby birds captured more often could be more likely to be seen with conjunctivitis. Finally, because multicollinearity in a model averaging framework can lead to biased parameter estimates with correlations as low as 0.55-0.65 [49], several pairs of variables (correlations > 0.55, see Table S1) were never included in the same models. However, results of model averaging were qualitatively similar if this cut-off was increased to a more liberal 0.7.

Because null hypotheses when using social network metrics are not necessarily ‘no effect’ [41, 50], we used randomisation tests to determine if parameter estimates for social network variables in top models (ΔAICc < 2) differed significantly from random. We used the asnipe package for R [43], where for each permutation step (1 to 10,000) the observations of individuals in two
different groups were swapped, creating an increasingly random network [51, 52]. We fit GLMs using the weighted degree or eigenvector centrality calculated for each random network with the same fixed effects for each of the top models, creating a distribution of coefficient values for these metrics [53]. If the coefficient in the observed network exceeded 95% of the coefficient values from the randomised networks, we considered this as support for a role of the tested metric in influencing disease risk.

II. Experimental Epidemics in Captivity

Experimental Design

Because our field data indicated that time spent on feeders was the most important predictor of super-receiving in this system (see results), we designed experimental epidemics in captivity to test whether time spent on feeders also predicted super-spreading. We quantified transmission dynamics in 10 single-sex flocks that differed only in the behaviour of the initially infected individual (Table S2). In half of the flocks, we initiated an epidemic by infecting the individual that spent the most time on the feeder (high-feeding index); in the other half, we infected the individual that spent the least time on the feeder (low-feeding index).

Field Captures, Transport, and Pathogen History Assessment

During March 2014, we captured house finches at two sites in and near Tempe, AZ. No birds showed clinical signs of Mycoplasmal conjunctivitis, which has not been reported in this region. Two weeks after arriving at Virginia Tech, all birds used in this experiment (n=44) were negative for anti-MG antibodies [54] (SM).
**Flock Housing**

In May 2014, birds were fitted with plastic leg bands and PIT tags, and transferred to single-sex flocks of four, in 76cm x 84cm x 46cm cages harbouring one tube-type feeder with one feeding port. Flocks of four regularly occur in the wild, particularly in the south-eastern US (mean winter flock size: 4-8), where MG epidemics have been explosive [26]. Feeder perches were equipped with an antenna and RFID receiver. Plastic sheets were draped between cages to minimize cross-cage transmission. Baseline data on feeder use were collected for seven days.

**MG Inoculation and Treatment Groups**

After two weeks in flocks, one bird per flock ("index") was inoculated in each conjunctiva with 40 μL of a solution of Frey’s medium containing 2 x 10⁵ Colour Changing Units (CCU) of MG, diluted from a stock described in SM.

Index birds were chosen based on the time they spent at the feeder during the prior week. We inoculated either the bird that spent the most time on the feeder (high-feeding index flocks) or the bird that spent the least time on the feeder (low-feeding index flocks). Because within-group variation in time spent on the feeder differed among flocks, we balanced our treatments across low-, mid-, and high-variance flocks (Table S2). Additionally, some flocks had consistent high-feeders, while others had consistent low-feeders. Because these flocks appeared at equal rates across low-, mid-, and high-variance groups, we inoculated low-feeders in flocks with consistent low feeders and high-feeders in flocks with consistent high-feeders (Table S2).
One flock of three birds and one single-housed bird were left uninfected to serve as sentinels for cross-cage transmission (Table S2). Due to the ethical and logistic limitations of keeping wild birds in captivity, the number of sentinels was low, but none showed detectable pathogen load or eye score.

**Monitoring the Experimental Epidemic**

One day prior to inoculation of index birds, and every 2 days post-inoculation (PI) until day 22 PI, all animals were captured for sampling. Sampling included eye scoring for clinical signs of infection (0-3 point scale described in SM), and conjunctival swabbing for quantification of MG load by qPCR, using previously published methods ([54]; see SM).

We used two methods to determine the initial transmission event within each flock. First, based on eye scores, initial transmission was defined as the first day on which any non-index bird showed eye score above 0. Second, because it is unknown whether transmission of MG can occur when birds are sub-clinical and because low false positive values can occur with our highly sensitive qPCR assay, we used two different qPCR-based cut-offs. These analyses defined initial transmission as the first day on which any non-index bird showed pathogen load above a given number of copies of the mgc2 gene. The first cut-off value was 0 copies; the second was 1,349 copies, which reflects an estimate of the minimum infectious load in this system (see SM).

**Statistical Analyses: Experiment Epidemics**

To determine whether birds that spend the most time on feeders act as super-spreaders, we tested whether transmission was more rapid in our high feeding index flocks using accelerated time
failure models with a Weibull distribution in the “survival” package [55] for R. This distribution makes minimal assumptions about the risk of transmission over time [56]. In addition, this distribution better fit the data than did other distributions tested (exponential, Gaussian, logistic, lognormal, loglogistic, or t), as assessed by ΔAICc > 2 [47]. To control for possible sex effects on transmission, we included sex as a covariate. In this analysis, the unit of replication was the flock (n=10).

To assess whether high- and low- feeding index birds were consistent in their feeding behaviours across time, we calculated repeatability using the intraclass correlation coefficient (ICC) in package ICC in R [57]. Finally, to test whether high- and low-feeding index birds might differ in other transmission-relevant metrics, we used general linear mixed effects models in package nlme in R [58] to test whether the two types of index birds displayed different levels of pathogen load or eye score across time.

**Results**

*Field Observations*

*Social network position.* We found limited support, in the opposing direction of our prediction, that social network position predicts risk of Mycoplasmal conjunctivitis. In particular, higher weighted degree was associated with a reduced risk of conjunctivitis. Weighted degree had a relative importance of 0.53 (Table 1; see Table S3 for the top 10 models), but the 95% confidence interval for this variable overlapped zero, suggesting a weak overall effect. However, among the top three models (ΔAICc < 2, Table S3), two included weighted degree and permutation-based p-values for weighted degree in both models were below 0.01. This suggests
that although the size of the effect may be small, higher weighted degree may be associated with reduced risk of conjunctivitis. We detected potential, but weak, effects of average adjusted group size and eigenvector centrality (relative importance of 0.34 and 0.33, respectively; both parameter estimates with 95% confidence intervals overlapping zero). Eigenvector centrality did not occur in any of the top three models (ΔAICc < 2), but did appear in two of the top ten models (Table S3). Although permutation-based p-values for eigenvector centrality from these models were both below 0.01, the signs of the parameter estimates were opposite, suggesting weak or inconsistent effects. Overall, results from social network metrics suggest that birds in larger groups may have had higher risk of Mycoplasmal conjunctivitis, while birds more central in the network may have had lower risk.

**Aggressive interactions.** We found minimal support for our prediction that birds engaged in frequent or diverse aggressive interactions are at higher risk for acquiring Mycoplasmal conjunctivitis. Each metric of aggressive contacts had a relative importance ≤ 0.25 and 95% confidence interval overlapping zero (Table 1).

**Foraging behaviours.** We found strong support for our prediction that foraging behaviours are important for disease risk in this system. The total time that free-living house finches spent on feeders per day positively predicted the probability of Mycoplasmal conjunctivitis, with a relative importance of 0.87, higher than any other variable (Fig. 1a, Table 1). Moreover, total time spent on feeders was the only variable whose 95% confidence interval did not overlap zero. Exclusion of the bird that spent the most time on feeders yielded nearly identical results (Fig. 1b).
Experimental Epidemics

When transmission was assessed using eye score, high-feeding index flocks, those in which the bird that spent the most time on the feeder was inoculated, showed a significantly shorter time to initial transmission than did low-feeding index flocks, in which the bird that spent the least time on the feeder was inoculated (Fig. 2a; overall model: $\chi^2_{df=2} = 11.69, p = 0.003$; treatment (low): parameter estimate = 0.48, $z = 5.42, p < 0.001$). This result held true when controlling for the effect of sex, as male flocks tended to have more rapid transmission than did female flocks (sex (male): parameter estimate = -0.64, $z = -3.06, p = 0.002$).

Differences in transmission between groups as measured by pathogen load were sensitive to the cut-off used. When using our best estimate of minimal infectious pathogen load (1,349 copies of the mgc2 gene, see SM), results closely match those obtained when defining transmission using eye score (Fig. 2b, overall model: $\chi^2_{df=2} = 9.39, p = 0.009$; treatment (low): parameter estimate = 0.35, $z = 2.70, p = 0.007$; sex (male): parameter estimate = -0.61, $z = -2.58, p = 0.01$). However, using a cut-off of zero copies of the mgc2 gene, no differences in transmission between groups were evident (Fig. 2c, overall model: $\chi^2_{df=2} = 0.68, p = 0.71$; treatment (low): parameter estimate = -0.05, $z = -0.50, p = 0.62$; sex (male): parameter estimate = 0.09, $z = 0.79, p = 0.43$).

Individual birds were consistent in their relative levels of feeder use both before and after inoculation (Intraclass Correlation Coefficient (ICC) (experimentally inoculated birds only) = 0.67, Fig. S2; ICC (including all birds) = 0.67, data not shown). In addition, high- and low-feeding index birds did not differ in the magnitude or time-course of eye score or pathogen load.
Discussion

Through observation in the field and captive experimentation, we found that individual variation in feeder use predicted both the likelihood of house finches acquiring Mycoplasmal conjunctivitis in the wild and the likelihood of transmitting the causative agent of this disease, *Mycoplasma gallisepticum*, in captivity. These results provide an example of a wildlife system in which the same behaviour makes an individual likely to be both a super-receiver and a super-spreaders. Such consistency in the traits underlying both exposure and transmission should favour more rapid and severe epidemics.

Consistent with prior work in this system [29, 59], our field results suggest that feeders play an important role in the risk of acquiring Mycoplasmal conjunctivitis. Hartup et al. [59] found that the presence of tube-style bird feeders was associated with an increased prevalence of Mycoplasmal conjunctivitis at the backyard scale. Our data illustrate a similar pattern at the individual level: the extent to which a house finch interacts with feeders in the wild predicts its likelihood of displaying Mycoplasmal conjunctivitis. These results are also consistent with Dhondt and colleagues’ [29] finding that feeders can act as fomites, facilitating indirect transmission of MG.

Although interaction with feeders was positively related to the risk of Mycoplasmal conjunctivitis in the wild, we found little evidence to suggest that aggressive interactions (a
proxy for direct contacts), or location within a social network, which could reflect direct or indirect contacts, were strongly predictive of disease risk. Consistent with our predictions and prior work [26], birds in larger groups tended toward higher risk for Mycoplasmal conjunctivitis, though the model-averaged parameter overlapped zero, suggesting a weak effect. Non-intuitively, we found that birds with lower weighted degree were more likely to be seen with Mycoplasmal conjunctivitis. Taken together, these metrics suggest that birds foraging in larger groups with unstable membership (low weighted degree, despite large sizes) are the most likely to acquire Mycoplasmal conjunctivitis, but the relative contributions of direct vs. fomite-based contacts to this pattern remain unknown. An alternative explanation for why birds with lower weighted degree were more often seen with conjunctivitis is that healthy individuals avoided diseased individuals. In this scenario, avoidance of sick birds would drive down diseased individuals’ weighted degrees and eigenvector centralities. While one study of captive house finches showed that healthy individuals actually prefer feeding near MG-infected conspecifics [60], another study found that finches can avoid birds actively mounting an inflammatory immune response [61]. Thus, avoidance of infected individuals cannot be ruled out.

Overall, our results suggest that time spent on bird feeders plays a more critical role in MG dynamics in wild birds than social network position or aggressive interactions. Constraints on our sampling regime might have limited our power to detect subtle effects of social network position on MG risk. Moreover, if behaviour away from feeders (e.g. at roosting sites) is unrelated to flock membership during foraging, our sampling may miss important social connections. However, prior work found that radio-tagged house finches roosting together at
night were more likely to be seen together during the day [62], suggesting that our social metrics should also reflect behaviours away from feeders.

Our field data lack continuous information on the disease status of all individuals, limiting our ability to assess causal relationships between feeding behaviour and disease risk. Although we physically captured approximately half of the birds in that study at least twice (n = 35 / 76), only three individuals changed disease status between captures. Thus, we lacked the resolution necessary to eliminate the possibility that post-infection behavioural changes altered the relationship between feeding and conjunctivitis. This remains an open research question in the study of disease dynamics in the wild. However, data from our captive experiment support time on the feeder as a cause (rather than a consequence) of increased risk of conjunctivitis: the feeding behaviour of inoculated individuals remained consistent prior to and following inoculation (Fig. S2). This pattern suggests that post-infection behaviour is unlikely to disproportionately influence the correlation between feeder use and disease risk in the wild, though more intensive study is needed on this topic.

While our field study shows that feeding behaviour predicts the likelihood of acquiring disease in the wild, our captive experiment demonstrates that feeding behaviour of infected individuals also predicts the likelihood of transmitting MG to susceptible flockmates. When transmission was assessed via visible pathology or the estimated minimum transmissible pathogen load, MG spread significantly more rapidly within flocks in which the inoculated bird spent the most time on the feeder. While differences in pathogen load or pathology, both of which alter pathogen deposition on feeders [63], could have influenced the pattern of transmission, neither factor
differed between high- and low-feeding index birds. Additionally, although dominance status correlated with time spent on feeders, it is unlikely that dominance per se resulted in more rapid transmission. In support of this idea, when models of time to transmission containing dominance were compared to models containing time on the feeder, the latter always showed lower AICc values (Table S4). Taken together, these results indicate that feeding behaviour is the most parsimonious driver of the observed differences in transmission rates between treatments.

Although behaviour has been hypothesized to impact disease dynamics in a suite of systems (e.g., [10-14]), this study provides rare empirical evidence that the behaviour of initially infected hosts can alter the trajectory of experimental epidemics in an ecologically relevant disease.

When we expanded our definition of transmission in captivity to include birds with one or more copies of the pathogen, we saw no differences in transmission between treatments. However, two factors suggest that such measurements are overly conservative. First, infectiousness (the ability to infect others) rather than merely harbouring a pathogen is the most relevant currency for transmission dynamics. Thus, assessing transmission using the estimated minimum infectious load should better capture the dynamics of our experimental epidemics. Second, two individuals were detected with loads of <50 copies of MG at only one time-point. These transient, low pathogen loads could reflect false positives in our qPCR assay or low pathogen burdens that were likely cleared before the bird could become infectious. In either case, counting these events as successful transmission likely overstates their importance in the epidemics.

Taken together, our field and captive results suggest that in this system, the same behaviour (feeder use) is important for both acquisition and transmission. These results highlight the importance of understanding relationships among behaviours that contribute to pathogen
acquisition and spread. Specifically, the pathogen’s potential for transmission should be highest, and epidemics most explosive, in systems with positive correlation between behaviours that predict acquisition and transmission [64]. In such cases, the highest risks of both acquiring and spreading a pathogen would be concentrated in the same individuals. For such systems, targeted disease management could be highly efficient and effective [10], as only one subset of individuals would need to be identified. In contrast, without correlation between the risks of acquisition and spread, similar management would require identifying two unique subsets of high-risk individuals, or focusing efforts on one class or the other, potentially limiting the efficacy of intervention. Further empirical and theoretical efforts are needed to describe such behavioural correlations and their impacts on disease dynamics across systems.

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References


46. R Development Core Team. 2014 R: A Language and Environment for Statistical Computing.


Table 1. Model-averaged parameter estimates and relative importance of variables in generalized linear models of the risk of Mycoplasmal conjunctivitis in wild house finches. Normalized model-averaged parameter estimates are displayed for visual comparison.

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<th>Model parameter</th>
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<td>N/A; contained in all models</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Number of captures</td>
<td>0.82 (-0.25 – 1.89)</td>
<td>N/A; contained in all models</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Time spent on feeders per day</td>
<td>0.17 (0.019 – 0.32)</td>
<td>0.87</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Weighted degree</td>
<td>-3.30 (-7.46 – 0.87)</td>
<td>0.53</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Average adjusted group size</td>
<td>21.90 (-28.54 – 72.34)</td>
<td>0.34</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Eigenvector centrality</td>
<td>-3.30 (-22.62 – 16.04)</td>
<td>0.33</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Relative dominance</td>
<td>0.55 (-4.33 – 5.43)</td>
<td>0.25</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Number of unique individuals w/ which interacted aggressively per day</td>
<td>-0.61 (-3.78 – 2.55)</td>
<td>0.24</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>-1.09 (-2.89 – 0.71)</td>
<td>0.18</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>(Unknown, n = 1)</td>
<td>-14.13 (-4,800 – 4,772)</td>
<td>[Diagram]</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Feeders visited per day</td>
<td>-0.61 (-10.60 – 9.39)</td>
<td>0.09</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Feeding bouts per day</td>
<td>0.10 (-0.020 – 0.23)</td>
<td>0.05</td>
<td>[Diagram]</td>
</tr>
<tr>
<td>Instances of aggression per day</td>
<td>0.44 (-0.14 – 1.02)</td>
<td>0.01</td>
<td>[Diagram]</td>
</tr>
</tbody>
</table>

-10  -5  0  5  10
Figure 1. Free-living house finches that spend more time on bird feeders are more likely to show Mycoplasmal conjunctivitis (a). Removal of the rightmost point yields nearly identical model predictions (b). Lines show predictions based upon top models from AICc model selection ± 1 standard error (shaded area).
Figure 2. In captive flocks of house finches, epidemics of *M. gallisepticum* progress more rapidly in flocks where the bird spending the most time on the feeder is experimentally inoculated (“high-feeding index” flocks, solid red line) than in flocks where the bird spending the least time on the feeder is inoculated (“low-feeding index” flocks, dashed blue line). When pathogen transmission is defined as the first occurrence of clinical signs (a) or the first detection of the estimated minimum infectious pathogen load (b) in an originally naïve animal, this pattern is highly significant. However, when transmission is defined as any detectable pathogen load, no differences between groups appear (c).
Supplementary Material

Methods

Overnight housing conditions

Birds included in the field study were single-housed overnight for a separate behavioral study in wire cages (76cm x 46cm x 46cm) at a constant 22°C, on a 12 hours light : 12 hours dark cycle. Birds were provided *ad libitum* food and water.

Estimate of local population size

We used the Lincoln-Peterson [1] method to estimate the local population size during winter. Briefly, we restricted our analysis to birds captured from November through February, as this period is most likely to minimize immigration and emigration due to seasonal migratory behaviors [2]. This time period was then divided into equal quarters. Based on these time periods, we generated three estimates of population size using pairs of consecutive quarters (*i.e.* Q1-Q2, Q2-Q3, Q3-Q4) according to the following equation:

\[
\text{estimated population size} = \frac{m_{i-1} \cdot n_i}{m_i}
\]

where \( m_{i-1} \) is the number of individuals marked during the prior quarter, \( n_i \) is the total number of individuals captured during the current quarter (both marked and unmarked), and \( m_i \) is the number of marked individuals captured during the current quarter. With four quarters, this generated three estimates of population size, which were then averaged to generate one estimate of 364 individuals.

Clinical signs of Mycoplasmal conjunctivitis as reliable indicator of infection in the wild
We used an “eye score” scale of 0-3 per eye to describe the severity of Mycoplasmal conjunctivitis, based upon previously published work [3]. Briefly, 0 indicates no detectable swelling or eversion of the conjunctiva, 1 indicates minor swelling and redness of the conjunctiva, 2 indicates moderate swelling and eversion of the conjunctiva, and 3 indicates severe swelling, with the eye nearly occluded. The conjunctiva of a subset of birds were swabbed for the presence of MG by qPCR (see below) during the 2012-2013 field season and a subsequent 2014 field season. We used all available swabs for birds with clinical signs of Mycoplasmal conjunctivitis (n = 22 swabs from 20 birds) and a random subset of birds without clinical signs (n = 74 swabs from 67 birds). The higher number of swabs than birds stems from recaptures of the same individuals.

**Quantitative polymerase chain reaction (qPCR) methods**

A sterile cotton swab was dipped into tryptose phosphate broth (TPB) and then rotated for 5s on the inside of each conjunctiva, using a separate swab for each eye. Both swabs were swirled in a single microcentrifuge tube containing 300µL of TPB and wrung out using the side of the tube. DNA was extracted from these samples using a Qiagen DNeasy 96 Blood and Tissue kit (Qiagen, Valencia, CA, USA). The amount of MG present was quantified by qPCR, using primers against the Mycoplasma mgc2 gene on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA), using previously published parameters [4, 5].

*Generating generalized linear models for use in model averaging*
As stated in the main text, variables correlated above 0.55 were not included in the same generalized linear models of disease in the field. Table S1 lists those correlations.

Table S1. Combinations of variables with Spearman correlations > 0.55 (indicated here by Xs).

These were never included in the same generalized linear models of field data.

<table>
<thead>
<tr>
<th></th>
<th>number of</th>
<th></th>
<th>feeding</th>
<th>feeders</th>
<th>time on</th>
<th>instances of</th>
</tr>
</thead>
<tbody>
<tr>
<td>average</td>
<td></td>
<td>individuals with</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted</td>
<td></td>
<td>which interacted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>group size</td>
<td></td>
<td>aggressively · d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>bouts · d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>visited · d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>feeders · d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>instances of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>aggression · d⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Experimental epidemics: initial capture and transport

After capture in and near Tempe, AZ, birds were brought to Virginia Tech by aircraft on March 8, 2014, and pair-housed in indoor cages (76cm x 46cm x 46cm) at 22°C, on a 12 hours light:12 hours dark cycle, with *ad libitum* food and water. After two weeks, a blood sample was
collected from the brachial vein and assayed for MG-reactive antibodies using previously published methods [5]. All birds in this experiment were negative for MG-reactive antibodies.

*Experimental epidemics: MG inoculation, treatment groups and qPCR*

Animals were chosen for experimental inoculation as described in the main text. Further experimental details are provided in Table S2, below.

MG inoculum was diluted from a stock solution of a MG isolate originally collected from an infected house finch in Virginia in 1994 [6] (VA1994, stock ID 7994-1-7P 2/12/09; D. H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh). This stock had a viable count of 2.24 x 10^7 CCU / mL [7]. For this captive experiment, we ran qPCR on only a subset of samples for pathogen load. For the index birds, we ran samples from pre-inoculation and on days 6, 12, and 18 post-inoculation in order to test whether high and low feeding index birds differed in their infectiousness. For originally naïve birds, we ran samples from pre-inoculation and days 4, 8, 12, 16, and 20 post-inoculation.
Table S2. Treatment groups for flocks in experimental epidemics. Each flock contained four birds, with the exception of the sentinel flock, which contained three birds.

<table>
<thead>
<tr>
<th>Flock number</th>
<th>Inoculation treatment</th>
<th>Sex</th>
<th>Intra-group variance in time on feeder (7d before inoculation)</th>
<th>Consistent low-feeder?</th>
<th>Consistent high-feeder?</th>
</tr>
</thead>
<tbody>
<tr>
<td>3(S)</td>
<td>Sentinel (randomly</td>
<td>Female</td>
<td>High</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Low-Feeder (MG)</td>
<td>Female</td>
<td>High</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>Low-Feeder (MG)</td>
<td>Female</td>
<td>Low</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>Low-Feeder (MG)</td>
<td>Male</td>
<td>High</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>Low-Feeder (MG)</td>
<td>Male</td>
<td>Mid</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>Low-Feeder (MG)</td>
<td>Male</td>
<td>Mid</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>High-Feeder (MG)</td>
<td>Female</td>
<td>High</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>High-Feeder (MG)</td>
<td>Female</td>
<td>Low</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>High-Feeder (MG)</td>
<td>Male</td>
<td>Mid</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>High-Feeder (MG)</td>
<td>Male</td>
<td>High</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>High-Feeder (MG)</td>
<td>Male</td>
<td>Mid</td>
<td>N</td>
<td>Y</td>
</tr>
</tbody>
</table>

Experimental epidemics: estimation of minimum infectious pathogen load

The detection of a pathogen within an individual does not necessarily indicate that individual can spread the pathogen to others. For example, low levels of pathogen may be cleared rapidly, resulting in no further transmission. Given this possibility, estimating the pathogen load at which an individual can spread pathogen to others (minimum infectious load) is highly germane to understanding infectious disease dynamics. We estimated the minimum infectious load of MG in house finches using previously published studies in this system. Briefly,
Dhondt et al. [8] showed that 42 days after inoculation, experimentally infected birds from the eastern US were no longer able to infect naïve birds, even by direct ocular inoculation. However, that study was conducted before methods to quantify MG load were available. We therefore used data from a different study involving eastern US finches [9], which used the same pathogen isolate as Dhondt et al. [8], to estimate the minimum pathogen load. Rather than use the mean pathogen load among eastern birds on day 42, we used the more conservative (i.e. lower) mean pathogen load on day 54 post-inoculation.

Experimental epidemics: effects of feeding vs. dominance

To further determine whether feeding per se, rather than social dominance, predicted an individual’s probability of spreading MG, we calculated relative dominance using data from RFID units in individual cages. As in the field, we counted the number of displacement events, defining these as 2s or less between subsequent detections of two different birds at the feeding port (see main text). We calculated dominance in two alternative ways. First, birds were assigned a dominance rank based upon their win:loss ratio, i.e. times displacing others:times displaced by others). Second, birds were ranked based upon the number of other birds they consistently displaced, i.e. the number of individuals a bird displaced more often than they were displaced by. Accelerated time failure models were then run using these dominance ranks in place of feeding preference (as was used in the main text) and compared via AICc.

Results

Clinical signs of Mycoplasmal conjunctivitis as reliable indicator of infection in the wild
In 93/96 (97%) samples assayed from the wild, results based upon clinical signs and qPCR generated the same conclusions regarding infection (i.e., infected or not-infected; mean square contingency coefficient \((\phi) = 0.91\), Fig. S1). Of the three cases in which metrics did not agree, all were low levels of detection: among birds without clinical signs, two samples showed approximately 4 copies of the MGC2 gene (0.56 and 0.63 on a Log\(_{10}\) scale, Fig. S1) by qPCR; among birds with clinical signs, one bird with an eye score of 0.5 showed 0 copies of MGC2 by qPCR. Overall, these results suggest that eye score is a robust proxy for infection.

**Figure S1.** Eye scores describing the severity of Mycoplasmal conjunctivitis (x-axis) positively predicted detection of *Mycoplasma gallisepticum* by qPCR (y-axis) in wild house finches (linear regression: \(r^2 = 0.80, p < 0.001\)). Closed circles on green backgrounds represent samples in which eye score and qPCR both indicated the same infection status (93/96); open circle on pink backgrounds represent samples in which these methods disagreed (3/96; mean square contingency coefficient \((\phi) = 0.91\)). Closed circles have been jittered slightly along each axis to better show individual points.
Statistical models of disease risk in the field

Table S3 shows the parameter estimates from the 10 best generalized linear models of the risk of conjunctivitis in the field, as calculated by ΔAICc.
Table S3. Top 10 generalized linear models of the risk of Mycoplasmal conjunctivitis in the field. The dependent variable in all models was being observed with conjunctivitis at some point during the study. All models used a binomial error distribution. The intercept and the number of times a bird was captured were included in all models. Only variables occurring in one or more of the top 10 models are shown.

<table>
<thead>
<tr>
<th>model</th>
<th>parameter estimates</th>
<th>AICc</th>
<th>weight</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>number of captures</td>
<td>time on feeders · d⁻¹</td>
<td>weighted degree</td>
</tr>
<tr>
<td>1</td>
<td>-3.69</td>
<td>0.89</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>-4.51</td>
<td>0.67</td>
<td>0.18</td>
</tr>
<tr>
<td>3</td>
<td>-4.92</td>
<td>0.91</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>-3.36</td>
<td>0.91</td>
<td>0.17</td>
</tr>
<tr>
<td>5</td>
<td>-3.57</td>
<td>0.90</td>
<td>0.17</td>
</tr>
<tr>
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<td>-3.65</td>
<td>0.93</td>
<td>0.17</td>
</tr>
<tr>
<td>7</td>
<td>-4.30</td>
<td>0.68</td>
<td>0.18</td>
</tr>
<tr>
<td>8</td>
<td>-3.27</td>
<td>1.00</td>
<td>0.19</td>
</tr>
<tr>
<td>9</td>
<td>-5.11</td>
<td>0.67</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>-4.60</td>
<td>0.68</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Experimental Epidemics

As discussed in the main text, high- and low-feeding index birds were consistent in their feeder use across time (Figure S2). Moreover, neither pathogen load nor eye score were different between high- and low-feeding index birds (Figure S3). Finally, the feeding behavior of the inoculated bird predicted the likelihood of transmission as well or better than did either measure of dominance (Table S4).

Figure S2. House finches experimentally inoculated with *M. gallisepticum* showed high repeatability in the daily time spent on a bird feeder prior to and following inoculation. Red triangles = birds that spent the most time on the feeder in a flock (“high-feeding index”); blue triangles = birds that spent the least amount of time on the feeder in a flock (“low-feeding index”). Error bars show ± 1 standard error.
Figure S3. When house finches were experimentally inoculated with *M. gallisepticum*, neither total eye score (sum of score for L and R eye) (a) nor pathogen load (b) differed between birds that spent the most time on feeders (“high-feeding index”) and birds that spent the least time on feeders (“low-feeding index”). Plots show group means ±1 standard error.
Table S4. During experimental epidemics, feeding behaviour of the infected bird explained the speed of transmission as well or better than dominance (measured either as an individual’s win:loss ratio or the number of other birds an individual consistently displaced).

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Independent Variables</th>
<th>AICc</th>
<th>ΔAICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to first transmission (eye score)</td>
<td>Feeding behaviour of infected bird</td>
<td>56.08</td>
<td>0</td>
</tr>
<tr>
<td>Time to first transmission (eye score)</td>
<td>Dominance (W:L ratio) of infected bird</td>
<td>57.00</td>
<td>0.92</td>
</tr>
<tr>
<td>Time to first transmission (eye score)</td>
<td>Dominance (# birds beaten) of infected bird</td>
<td>59.39</td>
<td>3.30</td>
</tr>
<tr>
<td>Time to first transmission (minimum infectious load)</td>
<td>Feeding behaviour of infected bird</td>
<td>56.66</td>
<td>0</td>
</tr>
<tr>
<td>Time to first transmission (minimum infectious load)</td>
<td>Dominance (W:L ratio) of infected bird</td>
<td>57.25</td>
<td>0.59</td>
</tr>
<tr>
<td>Time to first transmission (minimum infectious load)</td>
<td>Dominance (# birds beaten) of infected bird</td>
<td>58.52</td>
<td>1.86</td>
</tr>
</tbody>
</table>
References


Chapter IV: Feeder density enhances house finch disease transmission in experimental mesocosms

Co-authors: James S. Adelman, Damien R. Farine, Dana M. Hawley

*This article is in preparation for review in Philosophical Transactions B as part of a theme issue titled "Anthropogenic resource subsidies and host-parasite dynamics in wildlife."

Abstract

Anthropogenic food provisioning of wildlife can alter the frequency and intensity of intraspecific contacts, as well as contacts between hosts and environmental sources of pathogen transmission. However, despite the enormous popularity of backyard bird feeding, few studies have addressed how bird feeders influence host contact rates and disease dynamics. Here we experimentally manipulated bird feeder density in replicate outdoor aviaries and assessed effects on host behavior and pathogen transmission in a songbird disease system where feeders are important fomites of transmission. We varied the number of bird feeders available (2 vs. 4) to ten captive, pathogen-naïve flocks of house finches (Haemorhous mexicanus) of 8-9 birds. We then inoculated one bird per flock with Mycoplasma gallisepticum (Mg), a naturally occurring bacterial pathogen that is transmitted both via direct contact and indirect exposure at bird feeders. Birds were fitted with passive integrated transponders to continuously track foraging behaviors and social interactions at radiofrequency-identification equipped feeders. We found that pathogen transmission success was significantly higher in flocks with the highest density of bird feeders (4 feeders/flock), despite a significantly lower rate of intraspecific aggressive interactions relative to low feeder density flocks (2 feeders/flock). Conversely, amongst the naïve
birds that never showed signs of infection during the study, we saw significantly higher concentrations of Mg-specific antibodies in low feeder density flocks, suggesting that birds in low density flocks may have had exposure to subclinical of Mg. Finally, we did not detect any consistent differences in foraging behaviors across feeder density. Overall, our results suggest that the density of backyard bird feeders could play an important role in mediating the intensity of wildlife epidemics.

Keywords: Supplemental feeding, disease transmission, mycoplasmal conjunctivitis, *Mycoplasma gallisepticum*, house finch (*Haemorhous mexicanus*), bird feeders

Introduction

Anthropogenic resource provisioning of wildlife can occur either unintentionally or intentionally (Oro et al., 2013), and can alter disease dynamics in several host-pathogen systems (reviewed in Becker et al., 2015). Food provisioning can influence disease transmission via four primary mechanisms: increased density-dependent transmission due to changes in demography or altered contact rates among hosts, altered rates of contact between susceptible hosts and environmental sources of infectious agents, and changes in host condition and susceptibility (reviewed in Becker et al., 2015; Murray et al., 2016). For example, in white-tailed deer, supplemental feeding has been associated with higher prevalence of bovine tuberculosis via increased concentrations of deer and associated increases in density-dependent transmission (Miller et al., 2003). Similarly, supplemental feeding of elk results in higher brucellosis prevalence via increased direct contact rates and increased exposure to environmental sources of the pathogen (Cross et al., 2007). However, food supplementation can decrease the harmful effects of parasites and pathogens in some systems, largely due to improved nutrition (e.g. long-
tailed macaques and Giardia, Lane et al., 2011). Overall, while food supplementation has been broadly linked to disease outbreaks in several wildlife host taxa, there have been few experimental studies examining how resource provisioning alters disease transmission dynamics.

One of the most common forms of anthropogenic food supplementation is the use of backyard bird feeders. As of 2011, almost 1/6 of Americans were estimated to be actively feeding backyard birds (U.S. Department of the Interior et al., 2011). However, despite the enormous popularity of bird feeding, little is known about the health effects of feeder use on wild bird populations. Bird feeders have been hypothesized to facilitate disease transmission in several host-pathogen systems, yet causative links between bird feeding and disease prevalence remain exceedingly rare. In the only experimental study to date, Wilcoxen et al. (2015) manipulated bird feeder presence at forested sites and demonstrated a significant increase in infectious disease prevalence amongst wild birds captured at sites with supplemental feeding. Although increased rates of contact between hosts and/or hosts and fomites likely explains this result, Wilcoxen et al. (2015) did not quantify potential behavioral mechanisms. Likewise, since the mid-2000s European greenfinches and chaffinches have suffered severe epidemics of the protozoal parasite *Trichomonas gallinae*, and these epidemics are thought to have been exacerbated by the use of backyard bird feeders (Lawson et al., 2012; Robinson et al., 2010). *Salmonella* infections impact multiple species of songbirds, and are frequently tied to the use of backyard bird feeders (Tizard, 2004). Additionally, bird feeder use has been linked to the transmission of mycoplasmal conjunctivitis in house finches (*Haemorhous mexicanus*) (Adelman et al., 2015a; Dhondt et al., 2007), but to date there have been no manipulative studies examining how the density of bird feeders alter the dynamics of this host-pathogen system.
Mycoplasmal conjunctivitis, which is caused by the bacterial pathogen *Mycoplasma gallisepticum* (hereafter Mg), was first detected in house finches in the mid-1990s (Fischer et al., 1997; Ley et al., 1996). Since then, house finch populations in eastern North America have experienced annual epidemics of Mg during their non-breeding season (Altizer et al., 2004), the time of year when house finches forage in flocks and frequently visit backyard bird feeders. Mycoplasmal conjunctivitis in house finches is characterized by red and swollen conjunctiva, exudate, crusting around the eye, and depressed motor activity (Kollias et al., 2004). These clinical signs cause reductions in anti-predator behaviors (Adelman et al., 2017), leading to higher indirect mortality of diseased birds in the wild (Faustino et al., 2004). Mg can be transmitted via direct contact or indirectly via contact with contaminated environmental fomites such as bird feeders (Dhondt et al., 2007). Bird feeders can become contaminated with Mg when infected individuals stick their heads into feeder ports and deposit the pathogen via conjunctival exudate as they forage. The extent of time that an individual house finch spends on feeders has been positively linked to the likelihood of acquiring and transmitting Mg (Adelman et al., 2015a), suggesting that feeders are important for disease dynamics in this system. Additionally, the presence of tube type bird feeders in backyards has been linked to an increase in Mg prevalence in the wild (Hartup et al., 1998). However, elucidating the causative role of bird feeders for Mg transmission dynamics, as well as the mechanisms by which feeders alter disease spread, requires experimental manipulation.

In this study, we tested how the density of bird feeders influenced host behavior and the transmission of Mg within captive flocks of wild-caught house finches during experimentally induced epidemics. To test whether feeder density influenced feeding and/or social behaviors at feeders, we used radio-frequency identification device (RFID) equipped feeders to monitor the
foraging behaviors and social interactions of all flockmates, which were fitted with passive integrative transponder (PIT) tags. Since time spent on feeders was previously linked to Mg transmission (Adelman et al., 2015a), we predicted that a higher feeder density would result in increased access to feeder ports for individuals, resulting in more Mg transmission in high feeder density flocks. Specifically, we predicted that birds in high feeder density flocks would spend more time on feeders, and have longer feeding bouts than birds in low feeder density flocks which have to compete for limited access to feeder ports. Additionally, we predicted that the limited access to feeder ports within low feeder density flocks would lead to more aggressive displacements between birds at the feeder ports compared to flocks with a higher feeder density. We also tested whether the infectiousness of the “index” birds, which were inoculated with equal Mg doses to initiate the epidemic, differed for birds from high versus low density feeder treatments, which is a non-behavioral mechanism by which feeder density might alter Mg dynamics. Although food supplementation can influence host condition in several systems, including wild birds at backyard feeders (Wilcoxen et al., 2015), we did not expect “index” house finches in our study to show differences in infectiousness because our prior work has shown that time spent on bird feeders does not predict infectiousness (Adelman et al., 2015a) and that experimentally elevated levels of social competition at food sources do not alter infectiousness in this system (Adelman et al., 2015b). Finally, we measured body mass throughout the course of the epidemic because prior work indicated that feeder density alters body mass in house finches (Hawley et al., 2006). We predicted that birds with higher feeder densities would have higher body mass due to higher food intake and/or lower metabolic costs of intraspecific competition.
Methods

Experimental Design

To assess the effects of feeder density on social interactions and Mg transmission, we varied the density of bird feeders (high or low) within 12 captive flocks of house finches of equal size (n=6 flocks per density treatment; 1 flock from each treatment served as a sentinel flock). High feeder density (=HD) flocks had access to four feeders, each with one available feeding port, and low feeder density (=LD) flocks had access to two feeders, also each with one available port. All flocks were mixed-sex (3-4 females: 4-6 males) and initially consisted of nine randomly-assigned birds. However, mortality events during the acclimatization period left two flocks with only eight birds. We chose to remove one bird from two additional flocks before initiating the experiment, ensuring that we had an even number of eight- and nine-bird flocks across the two experimental treatments.

The single accessible port on every bird feeder was monitored by a radio frequency identification (RFID) device that recorded the time and duration of all feeder visits, along with the identity of the bird visiting the feeder. Food was provided ad libitum in feeders and refilled daily, and thus food was available at all of the feeder ports at all times. Our treatments thus did not differ in food availability per se, but rather competition for access to food resources. Within 10 experimental flocks, we initiated Mg epidemics by inoculating a single bird per flock (i.e. the "index bird") and tracked transmission for the following 27 days (Table 1). In our two “sentinel flocks” (one HD and one LD), a single bird per flock was sham treated with media alone (see below) and all flockmates were tracked as per experimental flocks. Because our prior work showed that the extent of time an index bird spends on a feeder, which is correlated with social status, predicts the extent of transmission in experimental epidemics (Adelman et al., 2015a), we
selected birds of intermediate social status from each flock as index birds (see *Inoculation* for details).

**Field Captures and Pre-Experiment Housing**

Hatch-year house finches (n=108) with no clinical signs of mycoplasmal conjunctivitis were captured June - September 2014 in Blacksburg and Radford, VA. All birds were caught using wire traps suspended around tube-shaped bird feeders or mist nets placed in close proximity of bird feeders. Immediately following capture, birds were fitted with an aluminum band stamped with a unique ID number and body mass was measured. Following processing and preceding the experiment, all birds were temporarily housed within an indoor animal care facility on the campus of Virginia Tech. For the first 19-24 hours, birds were held individually in small cages (46 x 46 x 76cm) in the same indoor room at a constant light cycle (12L:12D) and temperature (22-24°C), and provided with *ad libitum* food and water. After ~24 hours, birds were held in pairs in small cages (46 x 46 x 76cm), or in groups of four in larger cages (46cm x 76cm x 84cm), but all other housing conditions remained unchanged. Birds were monitored every 3-4 days during a 14-day quarantine period. Pre-experiment, any individuals that showed clinical signs of mycoplasmal conjunctivitis (swelling, redness, or exudate in either eye), or were ever housed with birds that showed visual signs of pathology were isolated and held in a separate room and were not used in experimental flocks. Additionally, the week before establishing experimental flocks, we screened all birds for Mg-specific antibodies (see below) and only birds seronegative for Mg exposure were used in the experimental flocks.

**Quantifying Mg-specific Antibodies**

We took a baseline blood sample from each bird to test for the presence of Mg-specific antibodies. Half of the birds were sampled on experimental day -21 (October 30th, 2014), and the
remaining half were sampled on day -20 (October 31\textsuperscript{st}, 2014). Blood samples were collected by puncturing the brachial vein using a 26-gauge sterile needle, and collecting blood using heparinized micro-capillary tubes. Blood samples were immediately stored on ice, and centrifuged within 4 hours of collection to extract plasma, which was then stored at -20°C. Enzyme linked immunosorbent assays (ELISA) for Mg antibodies as per Hawley et al. (2011) were performed using plasma extracted from these blood samples. We repeated this procedure at the conclusion of the experiment (day 27 - December 20\textsuperscript{th}, 2014) to assess whether individuals produced Mg-specific antibodies over the course of the experimental epidemics.

_Banding and PIT Tagging for Radio-Frequency Identification Monitoring_

Before moving birds into the aviary facility for the experiment, all birds were given a unique combination of color bands for visual identification, and fitted with a passive integrative transponder (PIT) tag. Each 0.1 g PIT tag was fastened to the color bands on the right leg using colored electrical tape matching the underlying band colors (as per Bonter et al., 2013). These PIT tags contained a unique 9-digit identifier that could be read by radio-frequency identification (RFID) equipped feeders. Each feeder used in the experiment had one accessible feeder port with a perch, to which an antenna was attached. Each antenna was connected to a reader which recorded data at a resolution of one data point per second (Adelman et al., 2015a). Readers logged the behaviors of PIT-tagged birds at the feeders uninterrupted from 06:00 to 19:00 for the duration of the study.

_Experimental housing_

On experimental day -17 (November 3\textsuperscript{rd}, 2014) all birds were moved to outdoor aviary compartments (5.5m x 2.5m x 2.4m), and grouped into experimental flocks. The aviary facility had a solid roof providing the birds protection from precipitation, as well as a cement foundation.
reaching approximately one meter in height. The internal half of the aviary compartments were enclosed with waterproof walls, while the external half of the aviaries were enclosed with heavy-duty zoological grade mesh exposing the compartments to ambient temperatures and natural light-dark cycles. Each flock was provided with four (0.46m long) perches made of wooden dowels, a synthetic Christmas tree, a heat lamp, ad libitum water in a plastic dish, and 2 tube-shaped RFID-equipped feeders. On experimental day -7, the six flocks assigned to high density treatment were provided with 2 additional feeders (Table 1) for the remainder of the study.

Inoculation

We used baseline behaviors from experimental days -7 to -1 to quantify dominance hierarchies based on aggressive displacements at feeder ports (see Extracting Behavioral Metrics Using Radiofrequency Identification Data for details). We used these metrics to select an index bird (= Mg-inoculated bird) of intermediate social status from each flock. This allowed us to control for underlying differences in transmission due to index bird access to feeders, which varies with dominance status (Adelman et al., 2015a).

On experimental day 0, we inoculated one index bird from each experimental flock with 70 μl of stock inocula containing ~2.46 x 10^8 color changing units of Mg diluted in Frey’s medium (2010.003-1-3P 10/25/2010; D.H. Ley, North Carolina State University, College of Veterinary Medicine, Raleigh). Inoculum was distributed approximately equally via micropipette across both conjunctiva. A single individual from each sentinel flock was sham inoculated with an equal volume of Frey’s medium alone.
Tracking Transmission

To track transmission, we sampled all birds for signs of infection every three days from day 0 through day 27 post-inoculation. During each sampling time point, we caught all birds using butterfly nets specific to that treatment group. To determine the presence or absence of visible pathology, all birds were given an “eye score” based on a 0-3 scale encompassing swelling, color, presence of exudate, and eversion of the conjunctiva (Sydenstricker et al., 2006). Eye scores were determined blind to treatment. To sample for the presence of pathogen, we swabbed each conjunctival sac using sterile cotton swabs. This entailed rotating tryptose phosphate broth (TPB)-saturated swabs along the inner conjunctiva for approximately five seconds, then swirling the swabs into a microcentrifuge tube containing $300 \mu l$ of TPB, and wringing out the contents of the swab on the inside of the tube. A separate swab was used for each eye, but the contents were pooled into the same microcentrifuge tube for a given bird and sampling day. We also recorded the mass for each bird at every sampling time point.

DNA was extracted from a subset of conjunctival swabs (days 0, 6, 12, 18) using Qiagen DNeasy 96 Blood and Tissue kits (Qiagen, Valencia, CA). We then used quantitative polymerase chain reaction (qPCR) to estimate abundance of MG in the conjunctiva using primers and a probe that target the mgc2 gene of MG (Grodio et al., 2008; Hawley et al., 2013). Standard curves of $2.98 \times 10^1$ to $2.98 \times 10^8$ copy numbers produced using a plasmid containing a 303 bp mgc2 insert were included in each run (Grodio et al., 2008).

Extracting Behavioral Metrics Using Radiofrequency Identification Data

Using RFID data, we extracted two general categories of behavioral metrics: foraging behaviors and aggressive interactions. We chose these categories because we hypothesized that bird feeder density would likely alter these behaviors, and they can predict disease transmission
in the house finches-Mg system (Adelman et al., 2015a). To test whether feeder density influenced the house finch foraging behaviors, we quantified the average amount of time individuals spent on all available feeders per day, the average length of feeding bouts, and the number of feeders birds were using relative to the number of feeders available to them. Aggressive interactions or displacements at the feeders were defined as any time two individuals were logged at the same feeder port within a two-second window of one another (Adelman et al., 2015a), indicating that a dominance interaction resulted in the rapid displacement of one individual by another. Pre-inoculation Elo scores were also calculated for each bird as a measure of relative social dominance. Elo scores integrate both the number of times a bird displaces or is displaced by another individual at the feeder as well as the identity and relative social status of the other individual (Neumann et al., 2011). We used Elo scores to identify birds of intermediate social status to use as index birds. Finally, we calculated what we term “following latency”, which is a metric pertaining only to flockmates, and is defined as the average length of time between an index bird departing a feeding port and a flockmate replacing it at the same feeder port.

Statistical Analyses

All statistical models were run in R (R Development Core Team, 2014) and models used a Gaussian distribution unless otherwise noted.

Feeder Density and Transmission Success

We used general linear models to assess relationships between feeder density and transmission success. Our response variable, transmission success, was defined as the number of transmission events divided by the number of naïve flockmates. We defined transmission events in two ways. Our primary definition of a transmission event was any time that a flockmate (non-
index bird) showed signs of visible pathology (=a non-zero eye score). Our second definition of a transmission event relied on qPCR results, which are subject to frequent low-level contamination (Leon et al., in review). Therefore, we used a conservative definition of infection established by Adelman et al. (2015): if a naïve flockmate had greater than or equal to 1349 copies of the pathogen present in the conjunctiva at any time point post-infection, we considered that as a successful transmission event. Our predictor variables for both transmission success models were feeder density and sex of the index bird.

Additionally, to assess relationships between feeder density and serum antibody concentrations of flockmates that did not show pathology throughout the course of the experiment (a measure of Mg exposure), we used general linear mixed models. We included flock identity as a random effect, and feeder density and sex as fixed effects. Our response variable was Mg serum antibody concentration. To assess relationships between feeder density and serum antibody concentrations of index birds, we used general linear models. Our response variable was Mg serum antibody concentration. Our predictor variables were sex and feeder density.

*Feeder Density and Disease Metrics in Index Birds*

We used general linear mixed effects models to assess relationships between feeder density and disease metrics in index birds. We included feeder density treatment, sex, and experimental day (Table 1) as fixed effects, and index bird identity as a random effect. Our response variables were conjunctival pathology (scores summed across both eyes and rounded to nearest integer, poisson distribution), pathogen load (log$_{10}$ transformed prior to analysis), and serum antibody concentrations at the completion of the study.

*Feeder Density and Behavior*
We used general linear mixed effects models to assess relationships between feeder density and behaviors of flockmates and index birds (analyzed separately). Behavioral data were separated out by week post-inoculation to account for any temporal effects of feeder density and/or infection. We included feeder density, sex, and week (week 0 = inoculation week) as fixed effects, and bird identity as a random effect. For models assessing flockmate behavior, we included flock identity as an additional random effect to account for the non-independence of flockmates. Our response variables were average time spent on the feeder per day (log$_{10}$ transformed prior to analysis), average feeding bout length (log$_{10}$ transformed prior to analysis), number of aggressive interactions, and following latency (flockmates only, negative binomial distribution). For the following latency model, feeder density and sex were included as fixed effects, and bird and flock identity as random effects.

**Body Mass**

We used general linear mixed effects models to assess relationships between feeder density and mass of flockmates and index birds (analyzed separately). For all models, we included feeder density, sex, and day as fixed effects, and bird identity as a random effect. For flockmates, we included flock identity as an additional random effect. Our response variable for these models was mass.

**Permits**

This study was conducted under the following permits: Virginia Tech Institutional Animal Care and Use Committee, Virginia Department of Game and Inland Fisheries (50352), and the United States Fish and Wildlife Service (MB158404-1).
Results

Feeder Density and Transmission Success

Feeder density treatment resulted in significant differences in Mg transmission success, as defined by the proportion of naïve flockmates that developed pathology (Feeder Density Low: $\beta=-0.14\pm0.05$, $F_{1,9}=8.50$, $P=0.023$; Figure 1). Specifically, Mg transmission success was significantly higher in flocks with high feeder densities. We found a similar, but non-significant pattern when we defined transmission success as the proportion of naïve flockmates that met the cut-off for “infected” established by Adelman et al. (2015a) (Feeder Density Low: $\beta=-0.059\pm0.034$, $F_{1,9}=3.00$, $P=0.13$). Sex of the index bird was not a significant predictor of transmission success in either model (Sex Male: $\beta<0.096\pm0.054$, $F_{1,9}<3.19$, $P>0.12$).

Overall, rates of transmission in our study were very low. Out of 91 naïve birds, only seven (7.69%) showed signs of pathology during at least one sampling time point, and only nine (9.89%), including the 7 with pathology, were considered infected using our pathogen load cut-off. Birds in the two sentinel flocks did not show any signs of pathology throughout the study.

At the termination of the experiment (day 27 post-inoculation), naïve flockmates that never showed any signs of pathology significantly varied in their Mg-specific antibody concentrations by treatment, with birds in low feeder density flocks having significantly higher antibody concentrations than birds in high feeder density flocks ($\beta=-0.011\pm0.0023$, $F_{1,68}=22.7$, $P<0.0001$; Figure 2).

Feeder Density and Disease Metrics in Index Birds

Index birds from high and low feeder density flocks had statistically indistinguishable levels of pathology and pathogen load over the course of infection (Pathology: $\beta=0.31\pm0.33$, $Z$-score=0.93, $P=0.36$; Pathogen load: $\beta=0.25\pm0.86$ $F_{1,39}=0.60$, $P=0.47$), indicating that index birds
from high feeder density flocks were not more infectious than those in low feeder density flocks. Index females had significantly lower eye scores than males (Sex F: $\beta=-0.84\pm0.38, Z_{1,49}=-2.23, P=0.026$), but sex was not a significant predictor of pathogen load ($\beta=-0.95\pm0.94 F_{1,30}=3.44, P=0.11$). Index birds from low and high feeder density flocks did not differ in their antibody concentrations at the conclusion of this study (Sex: $\beta=0.0067\pm0.015 F_{1,9}=0.20, P=0.67$; Feeder Density: $\beta=0.0051\pm0.014, F_{1,9}=0.14, P=0.72$)

**Feeder Density and Behavior**

**Daily Time on Feeders.** Feeder density had a week-specific effect on the average amount of time that naïve flockmates spent on feeders per day (Feeder Density * Week: $F_{1,379}=6.97$, $P<0.0001$). Post-hoc tests indicated that the only significant difference in the average time spent on feeders occurred in the week pre-inoculation (week -1), and in the opposite direction that we predicted: flockmates in low density feeder treatments spent significantly more time on feeders than those in high density feeder treatments (Tukey LS means: $P=0.014$, all post-inoculation weeks $P>0.17$; Figure 3). For index birds, we also found significant week-specific effects of feeder density on the average time on feeders per day (Feeder Density * Week: $F_{1,49}=2.74$, $P=0.046$). Index birds in high feeder density flocks spent significantly more time on feeders than birds in low feeder density flocks in week three post-inoculation (post-hoc Tukey contrasts LS means: week 3 $P=0.032$, all other $P$-values $>0.05$; Figure 3).

**Feeding Bout Length.** Feeder density treatment had a significant week-specific effect on the average feeding bout length of flockmates (Week*Feeder Density: $F_{1,377}=2.97$, $P=0.020$; Figure 4). However, post-hoc tests showed that average feeding bout length did not significantly vary by feeder density for any week during the experiment (post-hoc Tukey contrasts LS means: all $P$-values $>0.085$). For index birds, the average feeding bout length did not significantly differ
by feeder density treatment ($\beta=0.006\pm0.13$, $F_{1,49}=0.002$, $P=0.96$), but did significantly differ by week ($F_{1,40}=10.0$, $P<0.0001$; Figure 4). Sex was not a significant predictor of either average time spent feeding per day or average bout length for flockmates ($\beta=-197.67\pm260.33$, $F_{1,379}=0.58$, $P=0.45$) or index birds ($\beta=-0.40\pm0.23$, $F_{1,49}=2.87$, $P=0.13$).

**Relative Feeder Usage by Index Birds.** Index birds in both high and low feeder density flocks foraged at all feeders available to them throughout the experiment, with the mean proportion of total time spent on the most preferred feeder being 57.5% for low feeder density index birds and 42.1% for high feeder density index birds, and the mean proportion of total time spent on the least preferred feeder being 42.5% for low feeder density index birds and 9.2% for high feeder density index birds (Figure 5).

**Aggressive Interactions.** Feeder density had a week-specific effect on the number of aggressive interactions flockmates experienced at the feeder ports (Week*Feeder Density: $F_{1,349}=7.48$, $P<0.0001$). Consistent with our predictions, significantly more aggressive displacements occurred at feeder ports in low feeder density flocks than in high feeder density flocks, for all but the final week of the experiment (Tukey LS means: week 3 $P=0.29$, weeks -1-2 $P<0.003$; Figure 6). Index birds also had a significant week-specific effect of feeder density on aggressive interactions (Week*Feeder Density $F_{1,49}=3.18$, $P=0.027$). However, there were no significant differences in aggressive interactions across feeder densities for any weekly time spans (Tukey LS means: all $P$-values $>0.075$), perhaps due to the much smaller sample size of index birds relative to flockmates (Figure 6). Sex was not a significant predictor of the number of aggressive interactions individuals experienced (Flockmates: $\beta=0.065\pm0.097$, $F_{1,349}=0.44$, $P=0.51$; Index: $\beta=15.24\pm23.88$, $F_{1,49}=0.41$, $P=0.54$).
Following latency. The effects of feeder density on “following latency” (=time between an index bird leaving its position on a feeder port and a flockmate replacing it) varied with feeder density, with individuals in low feeder density flocks having shorter following latencies (β=-0.90±0.30, X^2_{1,19960}=8.91, P=0.0028) (Figure 7). Thus, birds in low feeder density flocks tended to feed at the same port as an index bird more quickly than birds in high feeder density flocks. Sex was not a significant predictor of following latency (β=0.076±0.081, X^2_{1,19960}=0.88, P=0.35).

Body Mass

Feeder density was not a significant predictor of body mass for flockmates (Feeder Density: β=0.061±0.39, F_{1,759}=0.0253, P=0.88), but day post-inoculation (Day PI) significantly predicted flockmate mass (Day PI: β=0.013±0.0019, F_{1,759}=11.2, P<0.0001). For index birds, feeder density had time-specific effects on body mass. Throughout the experiment, index birds in high feeder density flocks weighed more, on average, than index birds in low feeder density flocks, but the magnitude of this discrepancy varied with time (Feeder Density*Day PI: β=0.028±0.013, F_{1,99}=5.01, P=0.028; Figure 8). At capture, index birds later assigned to distinct feeder treatments did not differ in mass (Feeder Density High: β=0.53±0.87, F_{1,9}=0.37, P=0.56). Thus, the observed differences in index bird mass appear to be an effect of feeder density.

Discussion

We found that an increased density of bird feeders in replicated aviaries resulted in significantly more Mg transmission events (as defined by the presence of disease in naïve flockmates) during experimental epidemics. While previous studies have linked the presence of tube-type birds feeders with the prevalence of Mg in wild house finch populations (Hartup et al., 1998), this is the first study to experimentally vary access to feeders and examine effects on Mg
transmission. Additionally, we found that in the low density feeder flocks, in which had very low rates of detectable transmission were very low, individuals had significantly higher concentrations of Mg-specific antibodies at the termination of the experiment than birds in the high feeder density flocks. This discrepancy suggests that exposure to Mg may have been largely subclinical at lower feeder densities. Taken together, these results suggest complex potential links between the density of backyard bird feeders, Mg exposure, and Mg transmission success in free-living house finch populations.

Although we saw differences in transmission success between high and low feeder density flocks, we did not detect the predicted effects of feeder density on the foraging behaviors of house finches in our study. Feeder density did not consistently influence the amount of time spent on feeders, a trait previously linked to both acquisition and transmission of Mg (Adelman et al., 2015a) for either index birds or naïve flockmates. Index birds from high feeder density treatments did spend significantly longer average amounts of time on feeders in week 3 post-inoculation, which was consistent with our predictions and may partly explain the higher rates of transmission in flocks with high feeder densities. However, because differences in feeding behavior were only detectable during the final week of study, it seems unlikely that the amount of time spent on feeders was the primary driver of transmission differences in this experiment. Interestingly, index birds in high feeder density flocks maintained, on average, higher body mass than index birds in low feeder density flocks. This suggests that although we did not detect differences in time spent on the feeders between index birds across feeder density treatments, index birds at high feeder densities may have consumed more food than those at low feeder densities despite spending the same amount of time on the feeder. Higher feeding rates could potentially lead to depositing higher doses of pathogen while foraging. It is possible, however,
that some other physiological process (e.g. differences in metabolism or body mass regulation based on perception of food availability), rather than differences in food intake, drove this discrepancy in mass.

We also did not detect consistent treatment differences in the average length of feeding bouts, another behavior that could potentially influence Mg exposure or deposition at the feeders. The week prior to inoculation, naïve flockmates in the high density feeder treatment had longer average feeding bouts than birds in low density treatments, consistent with our initial predictions. However, this effect disappeared the week of inoculation, and thus differences in feeding bout length are also unlikely to be responsible for the observed discrepancy in transmission success. Overall, our foraging behavior results suggest that feeder density did not have strong and consistent effects on foraging behaviors in captive house finches, and thus, behavioral rates of contacts with feeders likely do not explain the detected treatment difference in transmission success. However, because our RFID approach detected merely the time spent sitting on feeders, it is possible that treatment differences in feeding efficiency and the rate of physical contact with fomites were present but not detected.

As we predicted, birds in low feeder density flocks engaged in more aggressive displacements at the limited feeder ports than birds in high feeder density flocks, with the exception of the last week of the experiment. These short-term indirect contacts at the feeders, and potentially direct contacts through the process of an agonistic displacement, did not, however, translate to higher Mg transmission success. In fact, we found significantly lower transmission success in the feeder treatment with the highest rates of aggression (e.g., low feeder density), indicating that in contrast to other wildlife disease systems (e.g. Hamede et al., 2013), aggressive interactions at the feeder are likely not important for transmission success in the
house finch-Mg system. Consistent with the higher levels of aggression detected in low density flocks, we found a significant effect of feeder density on following latency, with the shortest time spans between index birds leaving the feeder ports and flockmates replacing them occurring in low feeder density flocks. This is likely due to the fact that heightened competition in the face of limited resources leads to quick turnover in birds at the feeder ports. However, given that transmission rates were lowest in flocks with the highest following latency, it appears that closely following an index bird at the same feeder port is also not an important risk factor for Mg transmission.

The course of pathology and pathogen load in index birds did not differ across our feeder treatments, indicating that detected differences in transmission were not due to underlying differences in infectiousness. However, we unexpectedly found that flockmates in low feeder density flocks (excluding those that showed clinical signs) had significantly higher Mg-specific antibody concentrations at the conclusion of the experiment than flockmates in high density feeder flocks. This result suggests that there are potential feeder-density specific differences in physiology or exposure to the pathogen at or away from the feeders that we were not able to detect. It is possible that competition for access to feeder ports in the low feeder density flocks is increasing the likelihood of exposure to low doses of Mg that did not result in visible pathology. Perhaps the increased number of aggressive interactions that we detected in the low feeder density flocks led to exposure of small levels of Mg. Another possibility is that social stress surrounding competition for limited feeder access is causing a physiologically different response to similar levels of Mg exposure in the low feeder density flocks. However, serum antibody concentrations did not differ for index birds from high versus low feeder densities that were inoculated with identical doses of MG, suggesting that physiological differences are unlikely to
explain the detected differences across feeder density. Furthermore, we would predict the opposite effects of feeder density on antibody response based on prior work done in house finches. Using a similar experimental design, Hawley et al. (2006) injected birds at two feeder densities with equal doses of a non-pathogenic antigen (sheep red blood cells) and found lower circulating antibody concentrations in house finches at lower feeder densities, which is the opposite pattern from that detected here. Because exposure to the antigen was equal for all birds in that study, the authors interpreted these results as immune suppression due to the physiological stress of intraspecific competition at low feeder densities. Thus, the detected differences in antibody concentrations across feeder densities are most likely a result of differences in pathogen exposure dose. The behavioral mechanisms generating this difference, and the extent to which these small doses might provide meaningful immunological protection to individuals (Leon et al, in review), is an exciting area for further study.

One possible mechanism underlying the higher transmission rate detected in high feeder density flocks is simply the higher number of potential environmental sources of Mg (i.e., feeders) available. Conversely, higher feeder densities for equal flock sizes could lead to “dilution” of Mg deposition across feeders assuming index birds in both high and low feeder density flocks were depositing roughly equivalent copies of Mg on feeders. Unfortunately, we were not able to quantify the extent of Mg deposition on feeders because sampling the feeders would require removing Mg and potentially hindering transmission. We do know, however, that index birds in both treatments were using all of the feeders available to them. Thus, it is possible that high feeder density index birds were depositing Mg across more fomites and thus creating more potential environmental sources of Mg acquisition for flockmates.
Overall, our results suggest that bird feeders may play an important and potentially complex role in the dynamics of Mg spread in house finches, with a low density of bird feeders more likely to result in subclinical levels of exposure and a higher density of feeders more likely to cause ongoing transmission. However, we had very low transmission rates overall relative to past studies (Luttrell et al., 1998; Sydenstricker et al., 2006; Williams et al., 2014); thus, our ability to uncover the behavioral mechanisms involved in successful transmission was limited. Furthermore, while the captive nature of this study allowed us to directly manipulate feeder density and pathogen exposure, experimental manipulations of feeder density and presence in the wild are needed to better understand the role of feeders when birds are able to freely move across the landscape in response to changes in feeder density. Because wild house finches also have access to naturally occurring food sources, additional studies should investigate if and how Mg transmission dynamics differ in the presence and absence of bird feeders. Many backyard feeders have more than one feeder port, and social interactions across ports could lead to scenarios where aggressive interactions at feeders are decoupled from indirect short-term contact at specific feeder ports. House finches also regularly interact with other species of birds at feeders in the wild, potentially limiting the extent of indirect contact between sick and healthy house finches as other species may be picking up deposited Mg before susceptible house finches are exposed to it.

Overall, this study highlights the importance of the role of supplemental feeding on disease dynamics in a naturally occurring host-pathogen system. The fact that the magnitude of an experimentally induced Mg epidemic was influenced by feeder density in captivity suggests that the density and availability of backyard bird feeders could potentially play an important role in determining the extent of Mg transmission among free-living house finches. Further research is needed to determine the mechanism underlying variation in transmission success across feeder
densities. Additionally, future field based studies are needed to link feeder density with the prevalence and transmission of Mg in the wild. With more than 50.2 million Americans feeding birds (U.S. Department of the Interior et al., 2011), it is critical to understand the implications of backyard bird feeders on the health of common visitors, such as house finches.

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References


gallisepticum. J. Wildl. Dis. 40, 79–86. doi:10.7589/0090-3558-40.1.79


doi:10.7589/0090-3558-34.2.289


doi:10.7589/0090-3558-39.1.84


doi:10.1016/j.biocon.2016.10.034


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doi:10.1111/ele.12187


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doi:10.1053/j.saep.2004.01.008


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### Tables

**Table 1.** Experimental timeline for the study.

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
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<tbody>
<tr>
<td>-21-20</td>
<td>Blood sample for antibody concentrations</td>
</tr>
<tr>
<td>-17</td>
<td>Move to aviaries, establish flocks, begin logging behavior</td>
</tr>
<tr>
<td>-7</td>
<td>Establish experimental feeder densities</td>
</tr>
<tr>
<td>0</td>
<td>Pre-inoculation sample (mass, eye score, conjunctival swab), Inoculation</td>
</tr>
<tr>
<td>3, 6, 9, 12, 15, 18, 21, 24</td>
<td>Sample (mass, eye score, conjunctival swab)</td>
</tr>
<tr>
<td>27</td>
<td>Final sample (mass, eye score, eye swab, blood sample)</td>
</tr>
</tbody>
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Figures

Figure 1. High feeder density flocks (n=5) experienced significantly higher pathogen transmission success (number of naïve flockmates that showed pathology divided by the total number of flockmates) than low feeder density flocks (n=5).
Figure 2. At the termination of the experiment (day 27 post-inoculation), naïve flockmates that never showed any signs of pathology from low feeder density flocks had significantly higher antibody concentrations than flockmates in high feeder density flocks.
Figure 3. Flockmates in high feeder density and low feeder density flocks only differed significantly in the average time they spent on the feeder per day during week -1 (left). Index birds in high feeder density flocks spent significantly more time on feeders than birds in low feeder density flocks in week three post-inoculation (right).
Figure 4. Neither flockmates (left) nor index birds (right) differed significantly in the average length of their feeding bouts based on feeder density treatment.
Figure 5. Mean proportion of time index birds spent on their most to least preferred feeder.

Feeders were grouped based on preference, determined by the proportion of time each index bird spent at that feeder (i.e. a spectrum from most-preferred to least-preferred).
Figure 6. For flockmates, significantly more aggressive interactions (defined as two unique individuals logged at the same feeder port within two seconds of one another) occurred at feeder ports in low feeder density flocks than in high feeder density flocks, for all but the final week of the experiment (left). There were no significant differences in the number aggressive interactions across feeder densities for index birds (right).
Figure 7. Flockmates in low feeder density flocks had shorter average following latencies (=time between an index bird leaving its position on a feeder port and a flockmate replacing it) than birds in high feeder density flocks.
Figure 8. Index birds in high feeder density flocks weighed more, on average, than index birds in low feeder density flocks throughout the course of the experiment, but the magnitude of this discrepancy varied with time.
Chapter V: Synthesis

Life with pathogens and parasites is the rule rather than the exception, yet the vast majority of natural systems are characterized by heterogeneity among individuals in the likelihood or intensity of parasite infection. Variation in host behavior is an important source of this heterogeneity for many wildlife host-pathogen systems, as behavior has the capacity to impact a healthy host’s exposure to infectious agents, their physiological susceptibility to infection given exposure, and a sick host’s ability to transmit disease to healthy hosts (e.g. Barber et al., 2010; Dizney and Dearing, 2013; Ezenwa et al., 2016; Johnson and Hoverman, 2014). The work presented in my dissertation investigates behavioral and physiological variation in wild songbirds, the role of behavioral heterogeneity at bird feeders as it relates to disease acquisition and transmission, and the relationship between supplemental feeding of wild birds and disease transmission.

Animal behavior is not uniform across individuals, and one of the ways of describing this variation in behavior is animal personality. Animal personality, defined as a suite of correlated behavioral traits that are consistent across time and contexts (Réale et al., 2010; Sih et al., 2004), has been documented across many taxa including various invertebrates (e.g. Keiser et al., 2016), fish (e.g. Bell and Sih, 2007; Wilson et al., 1993), reptiles and amphibians (reviewed in Gosling, 2001), birds (e.g. Aplin et al., 2013; Drent et al., 2002; Snijders et al., 2014), and mammals (e.g. Best et al., 2015; Jin et al., 2013). In Chapter Two, I examined one of the most common measures of animal personality, exploratory behaviors (e.g Dingemanse et al., 2007; Drent et al., 2002; Sih et al., 2004; Verbeek et al., 1994). I found that free-living house finches vary in their exploration tendencies, and that more exploratory house finches interacted with more unique
individuals (had higher social network degree) in the wild. Such variation in social behaviors could serve as a mechanism through which variation in pathogen acquisition could arise, particularly for a directly-transmitted infection (e.g. Bull et al., 2012; MacIntosh et al., 2012; Vanderwaal et al., 2013).

While I found evidence that house finches showed variation in behaviors that could potentially lead to uneven exposure to pathogens in the wild, exposure does not lead to infection unless the individual is physiologically susceptible to disease. Zylberberg and colleagues (2013, 2014) found that house finches that exhibit riskier behaviors (e.g. associate with sick individuals, or exhibit more exploratory behaviors), invest more in immunological defenses against pathogens. In contrast, while I did not take immunological measures in this study, I found that more exploratory house finches had higher baseline concentrations of circulating corticosterone, a steroid hormone that has been hypothesized to suppress immune function (Apanius, 1998). Previous studies on house finches found higher concentrations of circulating corticosterone as a response to Mg infection (Lindström et al., 2005; Love et al., 2016); however further research is needed to investigate the role of corticosterone on susceptibility to infection upon exposure to Mg. Taken together, the results of these studies suggest that links between house finch exploratory behavior and physiology are likely not straightforward. Nevertheless, exploratory behaviors hold potential relevance to disease dynamics, as they can influence exposure to pathogens through interactions with conspecifics and the environment, and susceptibility to infection once exposed (via physiological differences). Further experimental studies directly linking exploratory behaviors to immune response, and how they ultimately relate to susceptibility to disease upon exposure to pathogens are needed.
The results of Chapter Two suggest that house finches vary in their behavior and physiology in ways that can influence both exposure and susceptibility to pathogens, however, I did not provide direct links between host behavior and disease. In Chapter Three, I investigated the relationships between host behavior and the acquisition and transmission of a common bacterial infection of house finches, *Mycoplasma gallisepticum* (Mg). Host behavior is known to be a source of heterogeneity in pathogen acquisition and transmission in many systems (e.g. Dizney and Dearing, 2013; Ezenwa, 2004; Fairbanks and Hawley, 2012; Fenner et al., 2011; Johnson and Hoverman, 2014), which can have important implications for the duration and intensity of disease epidemics (Lloyd-Smith et al., 2005). Variation in behaviors that influence pathogen exposure or transmission may be the basis of individuals contributing disproportionately to disease acquisition (“super-receivers”) and/or to disease transmission (“super-spreaders”). While previous studies have linked certain behaviors to super-receiving (Hamede et al., 2013) or super-spreading (e.g. May and Anderson, 1987; Shen et al., 2004; Temime et al., 2009), very few studies have provided evidence of a single behavior promoting both (e.g. Darbro and Dhondt, 2007). The results of Chapter Three provided evidence that the same behavior, time spent on a bird feeder, predicted both Mg acquisition and transmission, suggesting that time spent on the feeder can predict both super-receiving and super-spreading in this system. This behavioral covariation in both exposure and infectiousness could strongly influence the potential of epidemics, because the individuals most at risk of acquiring Mg are also those most effective at spreading it (Hawley et al., 2011). Identifying the behaviors and individuals likely to contribute to super-receiving or super-spreading could have important implications for disease management and wildlife conservation as it can be used to create more targeted management practices saving valuable time and resources (e.g. Rushmore et al., 2014).
As we saw in Chapter Three, interactions with anthropogenic food sources can have a strong impact on disease dynamics in house finches. Since feeder use was a predictor of both Mg acquisition and transmission, I further investigated the influence of bird feeders on the transmission of Mg. In Chapter Four, I tested whether bird feeder density influenced house finch behaviors surrounding bird feeders, and whether feeder density influenced Mg transmission in captivity. I found that while feeder density did not predict known transmission-relevant feeding behaviors, such as total time spent on the feeder, I saw significantly more Mg transmission in flocks that had more bird feeders available to them than in flocks with fewer bird feeders. Interestingly, while the two groups showed no difference in the amount of time that they spent on the feeders during the epidemic, Mg-inoculated birds (=index birds) in the high feeder density flocks were heavier on average throughout the course of the experiment than their low feeder density counterparts. Taken together, these results suggest that time spent on the feeder may not be an accurate proxy for time spent actively feeding, and underscore the need to further investigate mechanisms driving the link that I saw between time spent on the feeder and infection in Chapter Three. Transmission rates were low in this experiment (7.69%), and while I had a high resolution of behavioral data regarding feeder use and inter-individual interactions at the bird feeders, it is difficult to decipher any potential behavioral mechanisms of transmission. Future studies are needed to assess the influence of feeder density on transmission dynamics in the wild where birds can freely move across a landscape and between natural and anthropogenic food sources.

While identifying which individuals are driving transmission is important for management and conservation, disease is a natural process, and an important form of population regulation (Anderson and May, 1979). Thus, disease eradication is not the ultimate goal of
disease ecology. However, since human activity is increasingly influencing wildlife disease dynamics, it is important to understand the ways in which human activity might be exacerbating the effects of disease on wildlife populations. In Chapter Four, I explored how a very common form of human interaction with wildlife- supplemental feeding of birds - impacts the dynamics of Mg in house finches. Together, my results suggest that feeder use and feeder density are playing a major role in the acquisition and transmission of Mg. Use of non-tube-type feeders, and frequent decontamination of all feeders could mitigate feeder-related transmission of Mg and other pathogens. However, while bird feeders are linked to several avian diseases, they can be a valuable resource for birds in areas where natural food sources have been displaced or removed through urbanization.

In conclusion, I used both observational and experimental studies in field and laboratory settings to investigate the relationships among house finch behavior, anthropogenic food sources, and disease transmission. I provided evidence that house finches covary in their exploratory and social behaviors, as well as their physiology. I found that the extent of the use of bird feeders drives both Mg acquisition and transmission, making feeder use a predictor of both super-receiving and super-spreading. And lastly, I found evidence that the density of bird feeders available to house finches predicts the extent of Mg transmission. The results of Chapters Three and Four imply that indirect transmission at feeders plays a larger role than direct transmission in this system. Taken together, these results underscore the importance of behavioral heterogeneity in the acquisition and spread of pathogens as well as human impacts on wildlife behavior and in turn wildlife disease dynamics. Future work should seek to identify specific physiological mechanisms driving Mg acquisition and transmission as they relate to variation in host behavior, and the ways in which bird feeders impact these behaviors in the wild.
Wildlife are increasingly faced with anthropogenic changes as urban areas continue to expand, and human impact on the environment continues. Thus, better understanding the relationships between physiological and behavioral correlates in response to human activity, and how they relate to disease dynamics, can lead to valuable insights into the biology of wildlife in a changing world. Since Mg made its leap from poultry into wild house finches in 1994 (Hochachka et al., 2013), the house finch – Mg host-pathogen system has been an excellent illustration of the ways in which livestock, wildlife, and humans are interconnected. It is my hope that the work presented in this dissertation on the house finch – Mg system furthers our understanding of the impacts of behavioral heterogeneity and the influences of anthropogenic activity on wildlife disease dynamics.

References


doi:10.1093/beheco/arv003


doi:10.1016/j.anbehav.2013.08.003


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Fairbanks, B., Hawley, D., 2012. Interactions between host social behavior, physiology, and


