INFECTION DYNAMICS IN A HOST-PATHOGEN SYSTEM: HOUSE FINCHES (*CARPODACUS MEXICANUS*) AND *MYCOPLASMA GALLISEPTICUM*

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INFECTION DYNAMICS IN A HOST-PATHOGEN SYSTEM: HOUSE FINCHES (*CARPODACUS MEXICANUS*) AND *MYCOPLASMA GALLISEPTICUM*

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The transmission of parasites and pathogens among hosts remains an essential question in disease ecology. Transmission is rarely a simple process: variation among hosts, pathogens, the environment, and interactions of these factors can be crucial components in disease epidemics. In order to better understand the probability and rate that a pathogen spreads through a population, it is necessary to account for these multiple heterogeneities. House finches (*Carpodacus mexicanus*) are susceptible to a novel strain of the bacteria *Mycoplasma gallisepticum*, which causes conjunctivitis in the finches and other passerine species. I use both experimental work and long-term datasets on this host-pathogen system to better understand how both host and pathogen heterogeneities influence infection dynamics.

I find that individual behaviors can influence the risk of infection. Behaviors associated with indirect transmission, rather than direct transmission, increase infection risk. Also, the risk of infection is lower for socially dominant birds, but highly social infected birds can increase infection risk for susceptibles. However, the relative importance of indirect and direct transmission of *M. gallisepticum* is not yet clear. Viability of *M. gallisepticum* on antimicrobial feeders designed to kill the bacteria is not significantly different than viability on standard feeders, preventing a true exclusion of indirect transmission from this system. This demonstrates that

bacterial viability lasts longer than previously thought, further supporting feeders as an important source of *M. gallisepticum*. Bacterial load and distribution within and among house finch groups can predict infection probabilities, and these probabilities also show sex-based differences. Individual variation may also be responsible for the seasonal cycles of conjunctivitis: the introduction of naïve juveniles to a group of recovered adults causes an outbreak of *M. gallisepticum*, and reintroducing infected individuals to a group of multi-age, recovered individuals can also initiate a new wave of infections. Finally, community structure influences patterns of disease prevalence within house finches. Higher abundances of northern cardinals and American goldfinches were associated with higher disease prevalence in house finches. Taken together, these results show that heterogeneities within a host-pathogen system are essential to understanding how variation in infection patterns may influence disease dynamics.

BIOGRAPHICAL SKETCH

Sarah States was born on an unseasonably warm day on the 19th of March 1982 in Bath, NY. Growing up in rural upstate New York, she spent her time outside with her parents hunting down mushrooms, birds, wild ginger, and yellow ladyslippers. In high school, Sarah felt like she had finally found her place at the New York State Department of Environmental Conservation summer camps, where she learned about environmental studies and spent her time caving, canoeing, and backpacking in the Adirondack mountains. Despite her love for environmental science, Sarah decided to follow her other interests and major in music and theater when she attended Skidmore College in Saratoga Springs, NY. This stint as a theater major was unsatisfying and thus short-lived; Sarah switched to the environmental studies program, but still kept her attachment to music by minoring in voice performance. Sarah's first foray into biological research occurred as a technician for a project studying extra-pair paternity in savannah sparrows, working with Corey Freeman-Gallant. This opportunity piqued Sarah's interest in research, and after graduating she hiked all over Glacier National Park as a research technician on an amphibian population monitoring project. Deciding that she needed to broaden her cell biology experience, Sarah worked as a technician in an immunology lab at Tufts University in Boston, while always knowing that she wanted to return to ecology in graduate school. Sarah was thrilled to work at the renowned Cornell Lab of Ornithology, where she could pursue her interests in birds, ecology, and disease, a newfound interest.

I dedicate this dissertation to my mother, Rosemary States, for her unwavering and loving support, and to my father, David States, for inspiring my love of nature.

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CHAPTER 1

INTRODUCTION

Understanding how parasites and pathogens are transmitted among hosts is a vital question of disease ecology, and is crucial for developing measures to control disease dynamics. There are myriad factors that contribute to variation in the spread of parasites and pathogens ranging from the individual to community level. Variation intrinsic to the host, pathogen, environment, and the complex interactions among these factors complicate our efforts to explain differences in the probability and rates of transmission and to thus better understand and predict subsequent epidemics. Traditional epidemiological models (Diekmann and Heesterbeek 2000) simplified this complex process by subdividing the host population into discrete divisions or compartments that reflected the underlying disease state of the individual (Cooch et al. 2012). One such epidemiological model, the SIR model, partitions a host population into Susceptible (S), Infected (I), and Recovered (R) classes. In order for a disease epidemic to occur, individuals in the susceptible class must become infected at a particular rate, typically referred to as the force of infection, determined by the number of infectious individuals (I) within the population (Hudson et al. 2002).

Transmission in its simplest form is considered a mass action process, whereby the likelihood of infection of a susceptible host is a function of the proportion of infected hosts in the population and the frequency with which the susceptible individual interacts with these infected hosts (Heesterbeek et al. 1995). Two

interpretations of the mass action process exist:

1) Density-dependent transmission: Susceptible individuals make contacts at random with a fixed fraction of the individuals in the population.
2) Frequency-dependent transmission: Susceptibles randomly contact a fixed number of individuals, but only a fraction of these contacts lead to a new infection (Thrall et al. 1998).

Within both the susceptible and infected groups of the SIR and other epidemiological models, individuals are assumed to be part of a randomly mixing population, each with an equal probability of becoming infected. This assumption has been successful for many disease epidemics (Mollison et al. 1994), such as measles (Bjørnstad et al. 2002). However, in many epidemics, not all individuals are created equally, and we therefore must account for the heterogeneities present at various levels within host-pathogen systems. Variation within or between hosts due to physiological processes, behavior, parasite characteristics, environmental effects, or complex interactions of these factors all have the potential to affect the probability of infection or rate of pathogen transmission. Many studies have made headway in accounting for these heterogeneities in host-pathogen systems (Lloyd-Smith et al. 2005; Bansal et al. 2007; Böhm et al. 2009; Ames et al. 2011), but much remains to be done.

In this introduction I present background on the work done to explicate the roles of host, pathogen, and environmental heterogeneities that can influence disease dynamics at the individual, population, and community level. I then introduce a host-pathogen relationship that has been an informative system for exploring these heterogeneities. A novel strain of the bacterium *Mycoplasma gallisepticum* emerged as

a pathogen of house finches (*Carpodacus mexicanus*) in the 1990s and we have used field, lab, and captive work to study the complex host, pathogen, and environmental heterogeneities that drive patterns of infection and disease among individuals and subsequent effects on transmission at the population and community level.

Variation at the host level

Ultimately, the probability that an individual becomes infected is a function of both the probability that contact has been made with an infectious agent and the probability that such a contact will lead to successful infection (Begon et al. 2002). A variety of interrelated factors within these two criteria can determine whether a host becomes infected and then develops disease, and whether this host will be able to spread the pathogen to other susceptible individuals. Individual host variation due to genetics, physiology and behavior can play a key role in predicting a single host's contribution to the dynamics of infection and disease at the population level.

Probability of an infectious contact

Social animals have higher infection risks due to an increased number of close contacts with other infected conspecifics, which is related to the size and organization of social groups (Altizer et al. 2006). Group size alone may influence transmission: larger groups have higher contact frequencies, so directly transmitted parasites may be more abundant in larger groups (Cote and Poulin 1995; Clough et al. 2010). However, individuals within a social group can vary in their behavior, and this heterogeneity may have a large impact on the rate and probability of pathogen transmission (Bansal

et al. 2007; Fefferman and Ng 2007). Individuals with greater and more frequent contacts with other individuals are likely to transmit or become infected with pathogens (Lloyd-Smith et al. 2005; Böhm et al. 2009). This variation in contact rate is well known to have larger influences on population level disease dynamics in such cases as human sexually-transmitted diseases (Newman 2002), the recent SARS outbreak (Meyers et al. 2005), and bovine tuberculosis (Böhm et al. 2009). However, the intrinsic variation in host contact rates does not always have a significant effect on disease epidemics (Hamede et al. 2009).

Behaviors specifically associated with group living may affect transmission. Drewe (2010) found that meerkats who engaged more in grooming (but not receiving grooming) and those that roved between social groups were more likely to be infected with *Mycobacterium bovis*. Because the negative effects of parasitism can be high among social animals, behaviors that can reduce pathogen loads within group-living species should be favored. Some behaviors can act as parasite-avoidance strategies, such as allopreening, which reduces parasite loads (Radford and Du Plessis 2006), and roost switching to avoid ectoparasites (Reckardt and Kerth 2007). Social living may also facilitate age- or sex-based assortative mixing, which may constrain transmission patterns, as was the case of the H1N1 pandemic among school children (Cauchemez et al. 2011).

Social structures such as dominance hierarchies are common in group-living species, and can play a role in disease dynamics, and though a behavioral phenomenon, dominance status can affect stress hormones and immunocompetence (Cohen et al. 1997). Subordinate individuals of many species are often found to be

less immunocompetent than dominant individuals (Sapolsky 2004), but glucocorticoid levels may be elevated as a consequence of subordination or a cost of maintaining dominance status (Creel 2001). Experimental decreases in dominance status lead to reduced immunocompetence in house finches (Hawley 2006). Dominance status may likely influence transmission patterns among individuals, but the mechanisms of dominance and its relationship to host susceptibility to infection remain complex (Fairbanks and Hawley 2011).

Probability of successful infection

Parasites and pathogens can exert strong selective pressures on their hosts, and those hosts that can generate effective responses to the appropriate pathogens will likely be more successful. Despite these selective pressures, there can be wide variation in both susceptibility and response to infection within a host species. This variation may be the result of individual plasticity in immune response, but may also be due to genetic, immunological, or physiological factors (see reviews in Ardia et al. 2011 and Hawley and Altizer 2011). Host genetic variation can mediate pathogen susceptibility and can affect patterns of co-infection by multiple parasites and pathogens (Doums et al. 2000; Jolles et al. 2008). Host heterozygosity can predict responses to infection (Hawley et al. 2005), but more studies are identifying candidate genes that influence susceptibility to pathogens (Savage and Zamudio 2011). Genetic variation has the potential to drive among-host infection rates that could have consequences for population-level dynamics of infection and disease.

Sex-related traits can also constrain immune responses. While sex-based

behavioral differences may influence infection patterns (Bouwman and Hawley 2010), such patterns could be due to hormonal differences. Many studies of both humans and non-humans suggest that parasitic infections are often higher in males than females (Zuk and McKean 1996; Klein 2004). These studies indicate that immunological differences between the sexes underlie these differences in infection prevalence and intensity, and thus could have consequences for transmission. A hypothesis for this pattern is that the male hormone testosterone is immuno-suppressive (Folstad and Karter 1992). While this is not consistently the case (Roberts et al. 2004), experimental increases in sex hormones have been shown to increase transmission potential (Grear et al. 2009). Elevated testosterone levels were also associated with behavioral changes: red grouse *Lagopus lagopus scoticus* with higher testosterone levels also had higher nematode infections, occupied larger territories and were more likely to be bigamous (Seivwright et al. 2005).

Stress-related hormones can also have a strong influence on the immune response. Chronic stress, which can lead to long-term existence of increased glucocorticoids, can reduce immune function (McEwen et al. 1997). Individuals with elevated stress levels may not be able to initiate as an effective immune response as they would under normal conditions, resulting in trade-offs in immune investment (Jolles et al. 2008) or reduced immune function (Bartolomucci 2007). Individuals with lower immunological investment were more likely to develop high parasitemia (Beldomenico et al. 2009). Experimental reduction of corticosterone levels of mouse populations lessened the rate and magnitude of seasonal population declines, indicating that stress hormones can contribute to transmission dynamics within a

population (Pedersen and Greives 2008).

Variation at the parasite level

Within-host parasite dynamics can affect the likelihood that a pathogen will be spread within a host population. Variation in pathogen infection success depends on the ability of the parasite to respond to or evade the host's immune system, or within-host competition with other parasites or pathogens for resources (Tompkins et al. 2010). While the host immune system contributes greatly to the pathogen load and immunological response to the pathogen, variation among pathogen lineages, strains, or species can significantly affect transmission dynamics within the host population.

Variation on the part of the pathogen, such as replication rate and virulence, can affect the pathogen's abundance and distribution within a population of hosts (Ben-Ami et al. 2008; de Roode et al. 2008). Some pathogens, particularly macroparasites, show aggregated distributions among hosts, wherein a few hosts harbor the majority of helminths (Hudson et al. 2002), but similar distribution patterns are found in microparasite systems as well (Bertolino et al. 2003).

The consequences of pathogen variation for population-level infection dynamics can be great – high pathogen load individuals contribute more to infection rates within the host population (Lloyd-Smith et al. 2005). A study modeling the population-level effects of the fungal pathogen *Batrachochytrium dedrobatidus* on amphibian populations found that under high pathogen loads, host population extinction can occur (Briggs et al. 2010), indicating that knowledge of pathogen variation is vital to our understanding of transmission dynamics.

Environmental variation

Environmental conditions have the potential to alter transmission in a host-pathogen system. Temporal changes such as seasonality and temperature can drive population-level patterns of pathogen dynamics (Altizer et al. 2004; Savage et al. 2011). Environmental variability due to habitat and food availability can affect social aggregations and contact rates, potentially increasing contacts between infected and susceptible individuals (Robb et al. 2008). This may be particularly beneficial to parasites that cannot be transmitted directly between hosts. Social animals such as skinks (Godfrey et al. 2009) and lizards (Leu et al. 2010) indirectly spread parasites by sharing refuges. Where there is spatial overlap among hosts, such as in shared sleeping sites or refuges, parasites may occur in higher abundance (Bull et al. 1996). Pathogens may be able to take advantage of hosts that are densely packed in sleeping sites, as is suspected for the fungus *Geomyces destructans*, the causative agent of White Nose Syndrome (Lindner et al. 2011).

The presence of resource sites may facilitate indirect transmission as fomites, spreading the pathogen among susceptible individuals (Benskin et al. 2009). This is known for a number of pathogens of avian species, including *Mycoplasma gallisepticum* (Fischer et al. 1997), *Salmonella* spp. (Daoust et al. 2000), *Trichomonas gallinae* (Anderson et al. 2009; Lawson et al. 2011), and some mycotoxins (Oberheu and Dabbert 2001). Another indirect way in which pathogens may be transmitted is through environmental contamination. When the surrounding environment is contaminated with infected tissues, feces, or urine can contribute to transmission of

other susceptible individuals (Courtenay et al. 2006).

Environmental changes from anthropogenic disturbance can alter host-parasite systems, in turn affecting the probabilities of pathogen transmission. In Southern Cameroon, the prevalence of avian malarial parasites was higher in deforested habitats than in undisturbed areas (Chasar et al. 2009). Increasing urbanization has been shown to alter pathogen spread by affecting the distribution of both host and pathogen populations (reviewed in Bradley and Altizer 2007; Delgado-V & French 2012). Anthropogenic changes further push humans and wildlife into closer contact, which increases the likelihood of zoonotic disease transmission. The 1998 Nipah virus outbreak in Malaysia was thought to be caused by increased contacts between humans and fruit bats, the reservoirs of the virus, through agricultural practices (Olival and Daszak 2005).

Multi-species transmission

A large body of work has been devoted to understanding why some pathogens are generalists, capable of infecting multiple host species, and how this can drive disease dynamics within a community of hosts (Woolhouse 2001; Fenton et al. 2005; Keesing 2010). Host shifts, the transmission of pathogens from one species to another (often from wild species to humans, livestock, and domesticated animals) can have major consequences for these populations (Dazak et al. 2000; Cleaveland et al. 2001; Woolhouse et al. 2005). Indeed, pathogen 'spillback' to wild species can have major conservation implications, as is the case for mountain gorillas that are susceptible to human metapneumovirus (Palacios et al. 2011), or serve as a

mechanism of maintaining pathogens within the community (Kelly et al. 2009).

The presence of multiple hosts may reduce or amplify disease prevalence in a focal host, due to changes in host behavior in the presence of other host species, or a consequence of species-specific immunological differences. Heterogeneity among host species can also contribute significantly to pathogen spread: some species may act as superspreaders of a pathogen (Kilpatrick et al. 2006). Non-focal host species that are less competent as reservoirs may dilute the pool of available hosts, reducing the likelihood of transmission to the focal host (Keesing et al. 2006). In the case of Lyme disease, white-footed mice (*Peromyscus leucopus*) are the most competent hosts for the pathogen *Borrelia burgdorferi,* but when a higher number of host species are present, infection prevalence is lower with the vector (Keesing et al. 2009). It is also possible that the addition of other, more competent reservoir, non-focal species could amplify the pathogen prevalence in the focal host (Power and Mitchell 2004). For example, the North American bullfrog (*Rana catesbeiana*) is a reservoir of Bd in Brazil, spreading the fungus among other more susceptible host species, but rarely, if ever becoming diseased itself (Schloegel *et al*. 2009). Both scenarios exemplify ways in which the heterogeneities among hosts could alter the outcomes of the course of infection within a community.

Host behavior can facilitate between-species transmission, particularly behaviors that increase the likelihood of heterospecific contacts. This was evident following the badger-culling trials in Great Britain, an attempt to reduce the prevalence of bovine tuberculosis in cattle by removing the other primary host, the badger *Meles meles*. Inefficient culling actually caused badgers to roam farther

distances than badgers in unculled sites, inadvertently increasing the incidence of *Mycobacterium bovis* in cattle (Woodroffe et al. 2006). Heterospecific interactions serve as modes of transmission for pathogens that can be maintained in both hosts. *Brucella abortus*, a common bacterial pathogen of domestic cattle, was introduced to Yellowstone bison (*Bison bison*) in the early 20th century, and because of contacts between these two species, the pathogen is maintained in both species (Dobson and Meagher, 1996).

House finches and *Mycoplasma gallisepticum*

House finches and their recently emerged bacterial pathogen, *Mycoplasma gallisepticum*, have been a robust system for studying the many ways in which transmission heterogeneities at multiple levels can affect disease dynamics. House finches are native to the desert Southwest but were introduced to eastern North America in the 1940's (Elliot and Arbib 1953). Long a respiratory disease in poultry, a novel strain of *M. gallisepticum* emerged in house finches in the winter of 1993-1994 in a house finch in Maryland (Fischer et al. 1997; Ley et al. 1996). In the finches and other songbirds, the strain causes conjunctivitis and some rhinitis and sinusitis (Luttrell et al. 1998). The bacteria spread rapidly among house finch populations along the east coast, eventually spreading to the west coast (Dhondt et al. 1998; Dhondt et al. 2006). As a result of this epidemic, Eastern house finch populations showed regional population declines of up to 60% (Hochachka and Dhondt 2000). The pathogen is now endemic in finches, and exhibits a bimodal seasonal pattern of prevalence (Hartup et al. 2001; Altizer et al. 2004).

Among house finches, variation exists in patterns of infection and recovery, as well as host behavior associated with infection, and this variation contributes to population-level disease dynamics. House finches that are more heterozygous developed less severe infections than less heterozygous individuals (Hawley et al. 2005), though in experimental work, some individuals consistently remain uninfected (Sydenstricker et al. 2005) for the entirety of the study. House finches often recover from infection in the wild, but infections can sometimes be fatal (Luttrell et al. 1998). Dominant males recovered more quickly from infection and had lower disease severity than subordinate males (Hawley et al. 2007). House finches are highly social, and interactions with other individuals is considered a primary mechanism of transmission (Ley 2003). House finch house flocking behavior is thought to be responsible for patterns of increasing prevalence in the late fall (Hosseini et al. 2004).

Because *M. gallisepticum* has no cell wall, and can only survive 1-3 days outside its host (Christensen et al. 1994), indirect transmission is likely an important transmission mode in this system. There is strong evidence that feeders act as fomites, making them a critical component of pathogen transmission. We know that *M. gallisepticum*-inoculated feeders can infect naïve house finches (Dhondt et al. 2007), but results from observational studies suggest that certain types of bird feeders may contribute to conjunctivitis prevalence (Hartup et al. 2001). Resource use may alter behaviors that could contribute to infection patterns: diseased birds spend more time feeding (Hawley et al. 2007), and males, but not females, were more likely to feed close to diseased birds, presumably due to reduced aggression from sick individuals (Bouwman and Hawley 2010).

There have been strong seasonal fluctuations in prevalence, likely intertwined with variation in transmission rates. Across all areas where the epidemic occurred, there is a high peak in conjunctivitis in the late fall, followed by a minimum, and then another, smaller, peak in late winter (Dhondt et al. 1998; Altizer et al. 2004; Faustino et al. 2004). These patterns vary geographically in their exact timing and intensity (Altizer et al. 2004). The late fall peak is likely due to the seasonal influx of naive juveniles to the adult population, which may increase the probability of transmission from asymptomatic, but still infectious adults, while the late winter peak may results from the loss of temporary immunity and the stress of getting into breeding condition (Hosseini et al. 2004).

There is also evidence that this is not a single-host pathogen. Since early in the epidemic, other passerine species were observed with conjunctivitis (Hartup et al. 2000; Mikaelian et al. 2001; Farmer et al. 2005), though infection prevalence and competence of these other species as reservoirs was not yet known. Experimental work demonstrated that both American goldfinches (*Spinus tristus*) and house sparrows (*Passer domesticus*) both developed conjunctivitis and were infectious to house finches (Dhondt *et. al*. 2008). More recent fieldwork has found that many passerine species are often infected with the pathogen, but rarely develop clinical signs (J. DeCoste, pers. comm.).

The house finch - *M. gallisepticum* system has provided a rich opportunity to ask ecological and evolutionary questions regarding host-pathogen relationships. We have answered many questions, but there are still many areas of exploration. In my work I attempt to explain how host and pathogen variation contribute to infection risk

and evaluate the relative importances of transmission modes. These questions require exploration of variation at the level of host, pathogen, and environment to explain population- and community-level patterns of infection and disease. In Chapter 1 I focus on host behavioral heterogeneity to explain individual infection risk in captive groups of house finches. Previous work examined behavioral changes post-infection, but I attempt to predict how behavioral variation *prior* to infection can influence the likelihood that an individual becomes infected. I also parse out transmission-related behaviors associated with direct and indirect transmission to examine the relative importance of these two modes on infection dynamics.

My second chapter is a further attempt to explore the influence of transmission mode. I wanted to compare indirect and direct transmission modes, but it is difficult to isolate these modes in field or captive settings. Feeders made of antimicrobial materials, if capable of killing the bacteria, could potentially provide a method to experimentally remove *M. gallisepticum* transmission, creating an effective comparison of transmission modes. In this chapter I compare *M. gallisepticum* viability on antimicrobial and standard feeders through time.

In Chapter 3 I examine factors that drive temporal changes in infection dynamics within house finch groups. This experiment is the first experimental exploration of the importance of pathogen variation for house finch infection prevalence, taking advantage of variation across multiple captive groups to compare infection outcomes. Using multi-state mark-recapture models, I compare the relative influence of host sex, the pathogen abundance and distribution among hosts, and resource use on the rate of pathogen transmission, and assess the implications of these

results for population-level probabilities of infection.

Disease dynamics at the host population level are characterized by a large peak in disease in the late fall, and another smaller peak in the late winter. We suspect that these patterns could be driven by social aggregations of naïve juveniles and asymptomatic, but possibly still infectious, adults, but this hypothesis has not yet been tested. Using a long-term experiment with captive house finches, we test whether previously infected, but asymptomatic birds can cause a new epidemic among naïve juveniles, and if the reintroduction of an infected individual can spark a new epidemic. These results address necessary questions about population-level seasonal patterns we observe in wild house finches.

Finally, my fifth chapter addresses the importance of community-level variation in this host-pathogen system. Other passerine species vary in their infection prevalence, response to infection, and ability to transmit the pathogen, but we have not examined large-scale patterns of the influence of alternate host species on *M. gallisepticum* prevalence in house finches. Using citizen science data from Project FeederWatch and the House Finch Disease Survey, I examine whether other host species are associated with house finch disease prevalence.

My work addresses a variety of relevant questions that help elucidate how variation from the individual to the community level contributes to *M. gallisepticum* dynamics in house finches. I explore questions that show the importance of host, pathogen, and environmental heterogeneity, and that these traits ultimately have large consequences on host–pathogen relationships. I hope that this research may provide a basis for further exploration in this and other systems.

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CHAPTER 2

BEHAVIORAL VARIATION PREDICTS INFECTION RISK OF *MYCOPLASMA GALLISEPTICUM* IN HOUSE FINCHES (*CARPODACUS MEXICANUS)*

Abstract

Many population-level processes can be influenced by individual-level behaviors and interaction, including infectious disease dynamics. Traditional infectious disease models rarely account for the effects of inter-individual variation in behavior, but variation in host interactions may determine the degree to which an individual's behavior can negatively affect its health and the health of others in a group. We evaluate which of several host behaviors influence the risk of infection of a pathogen with multiple modes of transmission. House finches are host to the bacterium *Mycoplasma gallisepticum*, which causes conjunctivitis in many passerine species. Previous field studies and experiments using captive birds have demonstrated that house finches exhibit behavioral changes as a result of infection, but these studies did not identify how behavior prior to infection can influence transmission potential. Behavioral and disease data collected on three groups of experimentally-infected captive house finches allowed us to examine the influence of transmission mode, dominance status and sociality on the risk of *Mycoplasma* infection. We found that behavioral variation associated with indirect transmission rather than direct transmission had a greater influence on infection patterns. Additionally, the risk of infection for socially dominant individuals was lower than that for subordinate birds.

We also found moderate support for increasing likelihood of transmission if the source of infection is a highly social bird. These results indicate that a complex interplay of multiple behaviors influences the spread of infection within a group and that differences in individual-level behavior can dictate population-level disease dynamics.

Introduction

Traditional Susceptible–Infected-Recovered (SIR) models have long provided insight into the dynamics of infectious diseases (Anderson & May 1992; Grenfell 1992), but these models often fail to recognize the degree to which individual behavioral heterogeneity can affect pathogen transmission (Keeling & Eames 2005; Bansal et al. 2007; Fefferman & Ng 2007). Incorporating the complexity of social interaction into these models has allowed more detailed explanations of patterns of disease spread (Lloyd-Smith et al. 2005), but determining the relative importance of a multitude of seemingly relevant behaviors is still an arduous task, particularly in wild systems (Drewe 2010). Further difficulties arise when considering that some directly transmitted pathogens also can be indirectly, or environmentally, transmitted, and that some context-dependent behaviors of group living can influence pathogen susceptibility (Sarasa et al. 2009). In order to determine who is most susceptible, we need to identify and differentiate among multiple interactions that may have varying influences on transmission dynamics.

Because many pathogens have multiple routes of transmission, accurately predicting transmission may require knowledge about multiple types and rates of social behaviors that are potentially relevant to different transmission processes. Rates

of direct contact with infected individuals are well known to strongly influence disease prevalence (Alexander 1974; Bohm et al. 2008). Indirect contact with a pathogen through feeders or refuges can also influence the rate of pathogen transmission, as demonstrated in ibexes (Sarasa et al. 2009), skinks (Godfrey et al. 2009), and lizards (Leu et al. 2010). These studies demonstrate that indirect contact may be just as important as direct contact in pathogen transmission, but rarely have studies been able to distinguish between the relative importances of both transmission modes in a single host-pathogen system.

Variation in contact rates among group members is the most obvious mechanism by which an individual's social interactions can have potentially negative effects on its health and the health of others in the group. High levels of sociality could lead to two potential scenarios: a diseased individual may more easily spread a pathogen, and a healthy, susceptible individual may increase its likelihood of becoming infected (Sih et al. 2009). Complications in predicting the spread of infection arise when we consider that contact rates among group members are rarely uniform. In wildlife systems, as in humans, interactions are often age or sex biased, and disease spread may reflect these differential patterns of contact (Lusseau & Newman 2004; Cauchemez et al. 2010). Populations with highly skewed interaction rates may be more likely to have 'superspreaders,' individuals that play a disproportionate role in pathogen transmission leading to more explosive outbreaks (Lloyd-Smith et al. 2005).

Still other host heterogeneities such as dominance status and sex can affect population-level host-pathogen dynamics through influences on behavior and

immunocompetence. Subordinate individuals of many species have been found to be less immunocompetent than dominant individuals (Lindstrom 2004, Sapolsky 2004, Hawley 2006), probably due to the immunosuppressive effect of high glucocorticoid levels (Cohen et al. 1997). Though it is difficult to definitively state the role that dominance plays in immune suppression, these cases suggest that dominance status is an important but complicated factor affecting pathogen susceptibility. Sex differences in behavior (Grear et al. 2009) and pathogen compatibility (Sarasa et al. 2010) can play a role in transmission dynamics as well (Perkins et al. 2008).

A tractable system in which to study complex host-parasite dynamics is the house finch (*Carpodacus mexicanus*) and *Mycoplasma gallisepticum,* a bacterium that causes conjunctivitis in house finches and other wild birds (Ley et al. 1996; Hartup et al. 2001). In the winter of 1993-94, house finches in Maryland were observed with conjunctivitis caused by *M. gallisepticum* (Fisher et al. 1997), and since then the epidemic spread rapidly across the United States and Canada, resulting in regional declines in house finch populations of up to 60% (Dhondt et al. 1998; Hochachka & Dhondt 2000).

Prior work in the house finch – *M. gallisepticum* system has shown that both direct and indirect transmission of the pathogen can occur. Feeders can act as fomites, surfaces capable of transmitting pathogens, even though *M. gallisepticum* can only survive 1-3 days outside its host (Christensen et al. 1994). Captive experiments demonstrated that *M. gallisepticum* is infectious on feeders to susceptible house finches for up to 24 hours (Dhondt et al. 2007), indicating that indirect contact through feeders may contribute to infection. Infected house finches forage at feeders longer

and less efficiently than uninfected individuals, providing a greater opportunity for deposition of the bacteria and thus facilitating transmission (Hotchkiss et al. 2005). Given the low survival time of *M. gallisepticum*, direct transmission between hosts is also a likely mode of transmission (Ley 2003). The ability to recover from mycoplasmal conjunctivitis varies with sex and social status in house finches: dominant males recovered more quickly and had lower disease severity than subordinate males (Hawley et al. 2007b). This prior work suggests that multiple behavioral differences among individuals, such as feeding rates, rates of social interaction, and social status, can affect probabilities of becoming infected with *M. gallisepticum*.

In this study we use a hypothesis-driven framework to determine an individual's risk of *M. gallisepticum* infection by measuring interactions among house finches, before becoming infected. We collected behavioral and disease data on three groups of captive house finches, and differentiated among the interactions of infected and susceptible birds with other individuals and feeders. These data allowed us to quantify: 1) the amount an individual participates in behaviors associated with transmission mode (i.e. contacts with feeders or contacts with infected birds), 2) an individual's rate of social interaction with other birds, and 3) an individual's position in the dominance hierarchy.

With these behavioral parameters we tested, separately and together, the influence of behaviors associated with direct and indirect transmission on an individual's risk of becoming infected. However, because we believe that the roles of factors influencing susceptibility to infection are not mutually exclusive, we developed

four competing groups of transmission models that allowed us to determine the relative importance of both transmission-associated behavior and the potential additional effects of sociality and dominance status on an individual's risk of becoming infected. We also considered the possibility that no mode of transmission was entirely responsible for infection risk, so we also built a set of models that included single effects of dominance, sex, or sociality to account for the potentially strong effects of these other factors.

Previous work in this system explored changes in host behavior after infection occurred (Hawley et al. 2007a; Hawley et al. 2007b); our work is novel in that it 1) seeks to explain how behavioral traits characterized *prior* to infection may predict infection patterns and 2) examines how multiple behaviors may work in concert to affect the spread of one pathogen with multiple routes of transmission.

Methods

Experimental Setup

Juvenile house finches were caught using mist nets or wire mesh traps in Ithaca, New York, USA (42° 51'N, 76° 34'W) in late summer and fall 2007 under USGS Bird Banding Lab permit #23513. After capture, birds were banded with coloured leg bands for identification and housed individually for a minimum quarantine period of 2 weeks. Plastic dividers between all cages prevented interaction and potential indirect transmission during this period. During quarantine, birds were tested for presence of *M. gallisepticum* DNA by standard polymerase chain reaction (PCR) (Lauerman 1998), and for on-going or recent *M. gallisepticum* infection

indicated by antibodies using rapid plate agglutination (RPA) (Kleven 1998), and inspected for the presence of eye lesions typical of *M. gallisepticum* infections. Birds were tested within 2 days of capture, and again 1 week prior to the beginning of the study. Only birds that were negative for the bacterium in all tests were used in the experiment. To determine the sex of the birds we used a molecular-based PCR assay to amplify the sex-specific CHD-W and CHD-Z genes as in Griffiths et al. (1998).

We randomly assigned 33 hatch-year individuals in 3 separate flocks. Due to limited capture numbers, the sex ratio was female biased: there were 24 females, and 9 males. Each flock (8 females, 3 males) was housed in a large, free flight, semi-outdoor aviary composed of 2 peaked octagonal rooms connected by a corridor. Each octagon was 2.9 m in diameter and the sloped ceilings ranged from 2.4 m to 3 m high. The corridors were 1.5 m long × 2.4 m high and 1.2 m wide. Food was provided in clear plastic tube feeders with 6 openings (ports), each of which would allow only a single bird to perch and feed. There were 4 feeders and 1 water dish per aviary. Water and pelleted food (Roudybush, Inc., Cameron Park, CA, USA) were provided ad libitum. Artificial Christmas trees and wreaths were placed in the aviaries to provide perches and cover. All perches, water, and feeders were arranged identically among aviaries. Heating was provided near perches and water dishes using infrared lamps. Neither ambient temperature nor light cycles were altered; ambient noise levels were low.

On 25 February 2008, one bird from each flock was randomly selected as the index host from which *M. gallisepticum* would be transmitted to other flock members. These birds, 2 females and 1 male, were inoculated bilaterally in their conjunctiva with 0.05ml of *M. gallisepticum* stock inoculum (7th *in vitro* passage of the original

house finch *M. gallisepticum* isolate ADRL 7994-1; Ley et al. 1996) Index birds were held individually in a paper bag for at least five minutes to ensure absorption of the inoculum, after which these birds were returned to their aviaries.

To document disease status, every bird was trapped weekly until week 8 post-inoculation (PI) and then biweekly until week 14 PI. At each capture, we quantified disease severity by scoring physical symptoms in each eye on a 0–3 ordinal scale (see Sydenstricker et al. 2005). We also swabbed both conjunctivae to test for presence of the bacterium by quantitative PCR (qPCR) analysis (Grodio et al. 2008). Each eye swab was analyzed separately. An identical analysis was performed biweekly on choanal swabs. One week after inoculation and biweekly after that we took blood samples by venipuncture to test for *M. gallisepticum* antibodies using RPA.

By 1 week PI all 3 index birds were qPCR positive, developed bilateral conjunctivitis, and 2 of the 3 had seroconverted. The maximum eye score of index birds occurred at two weeks PI, when these birds all had a mean eye score of 2. All index birds remained qPCR positive for at least 6 weeks PI, and one still tested qPCR positive at week 8. Two birds retained eye scores of 1 until week 8 PI.

Ethical Information

All animal handling and maintenance procedures were approved by the Cornell University Institutional Animal Care and Use Committee (#2006-094). According to IACUC protocol, distress is defined as a bird that develops conjunctivitis, no longer can feed normally, and loses greater than 20% of its body mass. Signs of pain and distress were monitored through daily observation and health records. If a bird

appeared distressed or traumatized by their conspecifics, they were removed and house individually or euthanized. No pain relief medication was used in our experiment. Only euthanasia via a CO_2 chamber was used for alleviation of pain and distress.

Behavioral Observations

We observed all birds twice weekly from a blind for 1 hour between 0800 and 1200 hours. Observations began 3 weeks prior to inoculation on 4 February 2008 and continued for the duration of the experiment (14 weeks after inoculation). We recorded all independent contacts of birds with the feeder ports and dyadic interactions between individuals. The dyadic interactions included aggressive interactions (displacement of one bird by the other), close associations of two birds on perches (the two birds were no more than one bird-width apart), and courtship behaviors that included feeding and copulation. Whenever possible, both participants in the dyadic interactions were identified. When only one participant in an aggressive displacement interaction could be identified, this individual was still recorded as the winner or loser of the interaction.

Measures of interaction

In order to examine the influence of behavior on pathogen transmission, we parsed the observations into six types of behavioral parameters. Data on all behavioral traits used to predict the time until the first instance of infection among susceptible birds were collected during the weeks prior to inoculation of the index birds, hereafter

referred to as pre-infection observations. Thus, our analyses are explicitly intended to look for the influences of intrinsic behavioral characteristics of birds that affect their predisposition for acquiring disease, and not for the proximal effects of behavioral variation in the presence of diseased birds.

Data describing interactions between susceptible birds and the index birds refer to data collected prior to inoculation of these birds. Again, analysis of behavioral data collected in this way explicitly means our analyses will identify intrinsic characteristics of uninfected house finches that, however these traits are modified through disease in the index bird, are correlated with the probability of susceptible birds becoming diseased. We decided to use only data on behavioral traits described prior to any birds being diseased, because there was no other time at which to measure behavior that would clearly provide more relevant information. Due to a limited number of sampling periods, we could not determine when the actual transmission event occurred, whether transmission is a single event or a cumulative series of exposures of a susceptible bird to disease, nor if there was a consistent interval between transmission and the first manifestations of disease in susceptible birds. Effectively, any time at which to collect data on interaction rates was arbitrary, and our arbitrarily-chosen time provided the ability to make consistent biological interpretations of patterns identified from our statistical analyses.

Because there were unequal numbers of hours of behavioral observations made among aviaries and because we assume that the total number of contacts with another individual or feeder ultimately determines the likelihood of becoming infected, all behaviors, with the exception of dominance status, were calculated as rates: the total

number of times that a particular behavior occurred per hour. We explicitly define our six behavioral parameters in Table 1.

Data Analysis

All analyses were performed using R statistical software (see http://www.r-project.org). We used survival analysis (package 'survival'; Therneau 2012) to predict an individual's risk per week of becoming infected (i.e. we substituted "becoming infected" for "death," the typical response variable in these analyses) over the time of the experiment given our six behavioral parameters of interest. The first sign of infection was represented by qPCR data because they provide the earliest indication of *M. gallisepticum* infection. Cox proportional hazard models (Cox 1972) are particularly suited to this dataset because they allow us to estimate infection risk as a function of individual covariates. We calculated the hazard, h, which estimates the proportional increase in the weekly risk of susceptible birds becoming infected with *M. gallisepticum* over a baseline.

Our set of models can be viewed as describing four general processes of transmission, and each of the four model groups were structured in a similar manner (Table 2.2). The basic models were: behavioral modification of indirect transmission rates alone (*index feeder use* and *susceptible feeder use*), behavioral modification of direct transmission rates alone (*dyadic interactions* between a susceptible bird and its index bird), both direct and indirect transmission rates modified by behavior, or neither (the null model, with infection probability set to be constant, with no effect of any behavioral parameters). To each of these four basic models we subsequently

Table 2.1. Behaviors used to predict risk of infection by *Mycoplasma gallisepticum.* Each of the behavioral parameters on the left were calculated for the individual birds in the experiment through observations prior to infection. The second column describes how these parameters were calculated. These parameters are representative of a particular type of host heterogeneity (last column).

Behavioral Parameter	Description	Representative effect on infection risk
Dyadic	Measure of the rate of direct interaction between a susceptible bird and its index bird. Dyadic interactions are opportunities for transmission of *M. gallisepticum* directly between two individuals.	Direct transmission
Susceptible feeder use	Contact rate of a susceptible individual with all feeders. This is measured as the total number of times an individual visits the feeder.	Indirect transmission
Index feeder use	Contact rate of the index bird with all feeders. This is calculated as the total number of times the index bird visits the feeders, and is represented as one value per aviary. We assume that by including both susceptible and index feeder use together in our models, these rates provide the information needed to predict a susceptible individual's risk of becoming infected through the feeder, an indirect form of transmission.	
Dominance	Calculated using data on aggressive dyadic displacement interactions. The winner was the individual that successfully displaced the other participant from a feeder port or perch. The dominance score for each individual was calculated as the proportion of total interactions that an individual won. When it was impossible to identify both individuals in the interaction, the identifiable individual was assigned an 'unknown win' or 'unknown loss', depending on the outcome, which contributed to its dominance score.	Dominance structure
Susceptible sociality	Represents the rate at which each susceptible individual interacted with all other susceptible birds in its group. Calculated as the total number of interactions of a susceptible individual with all other susceptible individuals within its group.	Social interactions
Index sociality	Represents the overall interaction rate of the index bird with susceptible birds in its group. In order to keep this value independent of our measure of dyadic interaction rates (see above), the calculated value of index sociality for each susceptible bird excludes interactions between the index bird and that susceptible bird. Thus values of index sociality vary among all susceptible birds in an aviary.	

added the individual behaviors. *Dominance* status, an important, context-specific behavior, was added to all basic transmission models. Subsequently, we included *index sociality*, the variable representing the gregariousness of the index bird, to our models. Finally we added the *susceptible sociality* variable, but only to models that incorporated index sociality. This is because we thought that *susceptible sociality* would not have a large effect on transmission, but did not want to exclude a parameter representing the general gregariousness of susceptible birds.

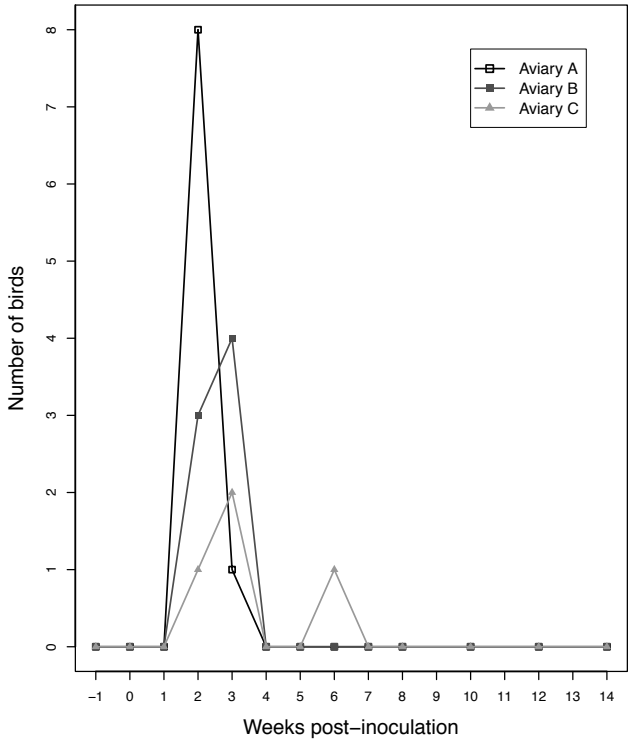
Table 2.2. The four transmission model groups (Indirect, Direct, Both modes, Neither). Each were structured in a similar manner. All contained the single transmission parameter only, and the other behavioral parameters were added sequentially. The basic null model (representing neither transmission mode) is set to a constant.

	Transmission mode (Indirect, direct, both, neither)			
1	Mode only			
2	Mode	+ Dominance		
3	Mode		+ Index sociality	
4	Mode	+ Dominance	+ Index sociality	
5	Mode		+ Index sociality	+ Susceptible sociality
6	Mode	+ Dominance	+ Index sociality	+ Susceptible sociality

This model group structure applies to all four of the transmission modes (for a total of 24 models). The "neither" model only model applies to the null model, and subsequent "neither" models refer to those in which only the behavioral characters of dominance and sociality are applied.

We used a hierarchical approach to find the optimal approximating model of

infection risk. We developed a set of candidate models and used Akaike's Information Criterion corrected for small sample sizes (AIC_c) to select the most parsimonious model (Lebreton et al. 1992; Burnham & Anderson 2002). We used the Akaike weights (w_i) as the probability that each model is the best model among the set of proposed models. The ratio of w_i between two models indicates the relative support between those two models. We calculated model-averaged coefficients for all



parameters from the entire model set (Burnham & Anderson 2002 pp.158-164).

Figure 2.1. Total new infections (qPCR positive) per aviary during each week of the experiment. Most infections occurred shortly after inoculation (week 0).

Results

Mycoplasma Infection

Overall, disease severity was low among susceptible birds that became infected. Across all flocks, 28 birds (93.3%) became infected based on all criteria for infection combined, though only 20 became qPCR positive and only 11 developed conjunctivitis. Because qPCR provides the earliest evidence of *M. gallisepticum* infection, and because seroprevalence was examined only biweekly due to sampling constraints, all further assignments of infection rely on qPCR data. Among aviaries, there were no significant differences in the proportions of initially susceptible birds that eventually became qPCR positive (two-tailed Fisher exact test, $P = 0.08$). Most of the infections in susceptible birds (qPCR positive) occurred shortly after inoculation of the index birds: 12 birds became qPCR positive 2 weeks PI, 7 at 3 weeks PI, and 1 bird at week 8 (Figure 2.1). The number of weeks individuals tested qPCR positive differed significantly by aviary (ANOVA: $F_{2,27} = 4.48, P = 0.021$). Individuals in aviary A were qPCR positive longer than in other aviaries (α=0.05, Tukey HSD). By the end of the experiment on 2 June 2008, all birds lacked signs of disease and were qPCR negative for *M. gallisepticum*, though 2 birds remained seropositive.

Variation in behavioral interactions among aviaries

We observed a total of 450 feeder visits during the pre-infection behavioral observations. Differences in behavioral parameters are shown in Table 2.3. There were no significant differences among aviaries in the contact rates of susceptible birds with the feeder (ANOVA: $F_{2,27} = 2.64, P = 0.09$). Likewise, among the index birds, there were no significant differences in their rates of feeder contact (ANOVA: $F_{2,21} =$

2.44, $P = 0.11$).

There were a total of 157 between-bird interactions of index and susceptible birds that were used to develop the dyadic interaction, susceptible sociality, and index sociality behavioral parameters. All correlations among behavioural parameters were nonsignificant. Among aviaries, susceptible birds were not significantly different in their rates of sociality (ANOVA: $F_{2,27}=0.523$, $P = 0.60$). There were significant differences in index bird sociality (ANOVA: $F_{2,27} = 1020.5$, $P < 0.001$), in that the aviary A index bird had significantly higher interaction rates than the index birds in aviaries B and C ($\alpha=0.05$, Tukey HSD). Similarly, susceptible birds

Table 2.3. Differences among aviaries in 5 of the 6 behavioral parameters calculated from observations. In two of these parameters, aviary A had significantly higher rates of contacts. Each value is calculated from the mean rate of behavioral interactions per bird during the baseline observation period. Index feeder use represents the daily contact rate of the index bird with its feeder. Dominance status is not included because it is context-dependent within aviary and cannot vary among aviaries.

	Aviary A	Aviary B	Aviary C			
Behavior	Mean±SE	Mean±SE	Mean±SE	df	*F*	*P*
Susceptible Feeder	1.97±0.30	1.60±0.16	1.29±0.13	27	2.64	0.09
Index feeder (rate indicates daily feeder use per index bird)	4.83±0.48	2.60±0.60	3.75±0.88	21	2.44	0.11
Dyadic interactions	0.63±0.12	0.11±0.08	0.10±0.04	27	12.68	**<0.001**
Susceptible sociality	0.90±0.18	0.70±0.12	0.82±0.11	27	0.52	0.60
Index Sociality	5.70±0.12	0.99±0.08	0.91±0.04	27	1020.5	**<0.001**

Table 2.4. Cox proportional hazard models ranked by change in AICc. We determined the risk of infection of *M. gallisepticum* for house finches based on six individual behavioral parameters. Behaviors were compared with four transmission models of increasing complexity. Letters represent the transmission mode group: indirect transmission (I), direct transmission (D), both modes (B), and neither (null) (N).

#	Model	K	AIC_c	ΔAIC_c	w_i
1	Index feeder + susceptible feeder + dominance (I)	4	112.654	0	0.222
2	Dominance + index sociality (N)	3	113.306	0.652	0.160
3	Index feeder + susceptible feeder + dominance + index sociality (I)	5	114.715	2.061	0.079
4	Index feeder + susceptible feeder + dyadic intxn + dominance (B)	5	115.265	2.610	0.060
5	Dyadic intxn + dominance + index sociality (D)	4	115.309	2.655	0.059
6	Dyadic intxn (D)	2	115.403	2.749	0.056
7	dyadic intxn + dominance (D)	3	115.446	2.792	0.055
8	dominance + index sociality + susceptible sociality (N)	4	115.925	3.270	0.043
9	index feeder + susceptible feeder (I)	3	116.394	3.740	0.034
10	dyadic intxn + index sociality (D)	3	116.439	3.785	0.033
11	index sociality (N)	2	116.951	4.296	0.026
12	index feeder + susceptible feeder + dyadic intxn + dominance + index sociality (B)	6	117.002	4.348	0.025
13	index feeder + susceptible feeder + dyadic intxn (B)	4	117.178	4.524	0.023
14	index feeder + susceptible feeder + dyadic intxn + index sociality (B)	5	117.441	4.787	0.020
15	dyadic intxn + index sociality + susceptible sociality (D)	4	117.818	5.164	0.017
16	index feeder + susceptible feeder + index sociality (I)	4	117.826	5.171	0.017
17	index feeder + susceptible feeder + dominance + index sociality + susceptible sociality (I)	6	117.864	5.210	0.016
18	dyadic intxn + dominance + index sociality + susceptible sociality (D)	5	117.975	5.321	0.016
19	index sociality + susceptible sociality (N)	3	118.352	5.698	0.013
20	dominance (N)	2	119.364	6.710	0.008
21	index feeder + susceptible feeder + dyadic intxn + index sociality + susceptible sociality (B)	6	120.196	7.541	0.005
22	index feeder + susceptible feeder + index sociality + susceptible sociality (I)	5	120.340	7.686	0.005
23	index feeder + susceptible feeder + dyadic intxn + dominance + index sociality + susceptible sociality (B)	7	120.439	7.784	0.005
24	Base null model (N)	1	121.251	8.596	0.003

in aviary A had higher dyadic interaction rates with their index bird than did susceptible birds in aviaries B and C (ANOVA: $F_{2,27} = 12.68$, $P < 0.001$, α=0.05, Tukey HSD).

Only aggressive dyadic interactions (N = 145) were used to calculate dominance status. Among infected birds, dominance status was stable between baseline observations and the status at time of infection was 0.76. ($T = 4.95$, $P < 0.001$).

Model results

Among 24 candidate models, only 2 had appreciable support from the data; both had $w > 0.100$ (Table 2.4). The top model, (w=0.222) included the parameters representing indirect transmission (index feeder use and susceptible feeder use) and dominance status. The second-best model, part of the null model group, included index sociality and dominance status, and received a weight of 0.160. The third and fourth models, with $w > 0.050$, are more complex but still reinforce that behaviors associated with indirect transmission have greater influence on infection risk. The basic null model was lowest-ranked in our candidate model set. We do not report models with sex as a parameter because in all cases, models with sex had reduced support relative to models with all other parameters the same but with the absence of sex as a parameter.

Do transmission-associated behaviors affect the risk of infection?

To evaluate the degree to which behaviors related to transmission modes might

influence infection risk, we compared relative model support across equivalent transmission model groups. The top model included indirect transmission-associated behaviors, and was 3.69 times better than the equivalent model representing both transmission modes (#4) and 4.04 times better than the equivalent direct transmission model (#7). The best-performing model in the direct transmission group included dominance and index sociality and was ranked #5, but the top model still received 3.77 times greater support. Higher rates of contact with feeders and higher rates of dyadic interactions were all associated with higher probabilities of infection. Model-averaging yielded a positive effect of index and susceptible feeder use on infection risk (ß=0.05, 95%CI=-0.53-0.64 and ß=0.34, 95% CI=-0.11-0.79, respectively) (Table 2.5).

Table 2.5. Parameter estimates, unconditional standard error, and upper and lower 95% confidence intervals based on model-averaged estimates across all models. β represents the regression coefficient.

			95% CI	
Parameter	β	Unconditional SE	Lower	Upper
Dominance status	-12.23	6.524	-25.021	0.553
Dyadic interaction	0.46	0.459	-0.437	1.361
Susceptible sociality	-0.03	0.074	-0.176	0.116
Index sociality	0.18	0.149	-0.111	0.473
Index feeder	0.05	0.298	-0.534	0.636
Susceptible Feeder	0.34	0.231	-0.112	0.793

While our analyses suggest that indirect transmission is the most important mode of transmission, examination of our results (Table 2.4) also indicates that

predictors of transmission mode by themselves cannot fully explain our observations. Two factors unrelated to transmission mode, dominance and index sociality, were included in the top two models. In comparison with other transmission mode models containing both dominance and index sociality, we found that the null transmission model of dominance and index sociality (#2) received the most support. This model was 2.02 times better than the equivalent indirect transmission model (#3), 2.72 times better than the direct transmission model (#5), and 6.35 times better than the equivalent model with both transmission modes.

Further supporting the conclusion that indirect transmission alone is not sufficient to explain our data, we found that the indirect transmission model including dominance (#1) had 6.49 times greater support than the indirect transmission-only model (#9).

Does dominance status influence the risk of infection?

Dominance was strongly supported as a behavior influencing individual infection risk. Not only is dominance found in the top 5 models, but when comparing the top-performing models of increasing complexity to their equivalent models, those that included dominance always had greater support. The top model (#1) that included dominance performed 6.49 times better than the equivalent model that excluded dominance (#9). The second-highest ranked model, which included dominance and index sociality, was 6.18 times better than the equivalent model that excluded dominance (#11). Finally, when comparing the top-performing model that includes all three additional behavioral parameters (dominance, index sociality, and susceptible

sociality — #8), the equivalent model excluding dominance (#19) received 3.38 times less support. We model-averaged across all candidate models and found a strong pattern of higher dominance being associated with lower infection risk (ß=-12.23, 95%CI=-25.02-0.55) (Table 2.5).

Is sociality of the index or susceptible bird important for predicting the risk of infection?

We found moderate support for an effect of the sociality of the index bird on infection risk in our system. The parameter was part of the second-best supported model, which also included the effect of dominance status. This model was 20.79 times better than the equivalent dominance-only model (#20). For all transmission-only models, the addition of index sociality reduced support; however, support increased when the effect of dominance was added.

None of the models including susceptible bird sociality were well supported; they received weights below 0.044. The model-averaged estimate for index sociality indicated an increasing hazard with higher index contact rates (ß=0.18, 95%CI=-0.11-0.47). However, contrary to our a priori expectations, the estimate for susceptible bird sociality indicated a decreasing hazard (ß=-0.03, 95%CI=-0.18-0.12).

Discussion

Our data show that predicting infection by *M. gallisepticum* is a complicated process; no single factor alone could explain all variation seen in infection probabilities within or among our house finch groups. However, we found

considerable support for the effects of behaviors that most influence indirect transmission and dominance status on infection risk of *M. gallisepticum*, and moderate support for sociality of the index bird. As hypothesized, increased contact with the feeder increases the risk of an individual becoming infected. There was a strong negative effect of dominance status, indicating that individuals of higher status are less likely to become infected. Finally, the sociality of the index bird influenced patterns of infection: when diseased birds have high contact rates with other individuals, susceptible birds are at greater risk of becoming infected.

Direct or indirect transmission and risk of infection

A long-standing interest of our work has been to differentiate among the relative importance of direct and indirect transmission routes for the spread of *M. gallisepticum* (Dhondt et al. 2005). Dhondt et al. (2007) experimentally demonstrated that feeders are an indirect source of the pathogen, capable of infecting birds for up to 24 hours. Likewise, the pathogen is thought to be spread directly between individuals (Ley 2003), and given the gregarious nature of house finches, this is a logical assumption. No direct comparison of these routes has yet been done, but our analysis of the degree to which individual behaviors associated with transmission patterns moves us closer to this goal. Our top model — with indirect transmission — was more than 3.5 times better than the equivalent transmission models that contained direct transmission only, or both direct and indirect transmission. This provides strong support that individual behaviors associated with transmission through the feeder play a stronger role in the risk of infection of *M. gallisepticum* than direct transmission.

This work implicates the important role that bird feeders likely play in this system; wild house finches are gregarious and frequently congregate at feeders. Behaviors of diseased house finches may exacerbate the spread of infection: in both the wild and captivity, previous work documented that diseased birds spend more time at the feeder (Hawley et al. 2007a), potentially depositing bacteria that can be picked up by susceptible individuals. Many other bird species harbour the bacterium, often without developing symptoms (Hartup et al. 2001; Dhondt et al. 2008). Even though the opportunity for interspecies contact is less than for intraspecies contact, the stronger influence of indirect transmission of *M. gallisepticum* through feeders would allow the pathogen to circumvent this problem. Furthermore, among the East coast strains that have emerged and been isolated, we have seen evidence for increased pathogen virulence through time, with these more virulent strains tending to produce higher bacterial loads (Grodio et al. 2012). If more virulent variants of the pathogen produce higher bacterial loads, or survive longer on the feeders, transmission through feeders could be a potential explanation for increased virulence through time or space.

An earlier experiment using individually-housed birds first determined that feeders do act as fomites for *M. gallisepticum*; however, disease severity was low, reducing the likelihood of a large outbreak (Dhondt et al. 2007). Here disease severity was higher than in the previous experiment even with the same isolate of *M. gallisepticum*, and this could result from: 1) a threshold effect whereby infected birds constantly reinfect the feeder, increasing the likelihood or severity of infection in susceptibles, 2) direct contact, and thus transmission, between infected and susceptible birds playing a role, 3) susceptible birds experiencing additional exposure to *M.*

gallisepticum through feeding on the floor or contacting other common surfaces such as perches, or 4) resistance of birds being compromised in a group setting where they have to compete for food (Hawley et al. 2006). Because behavioral traits associated with indirect transmission alone did not explain all the variation in infection risk, we do not deny the role that direct transmission may have in infection patterns; indeed, the presence of feeders themselves may facilitate these interactions to some degree. We also cannot rule out the possibility that other fomites such as branches or perches could also act as an indirect source of *M. gallisepticum*, though feeders are likely the most concentrated source of the pathogen, especially in the wild. We did not measure immunocompetence in this study; however, in our aviaries there was relatively low competition for food, (4 6-port feeders per 11 birds) making it unlikely that competition alone reduced resistance to *M. gallisepticum*. Given these results, we conclude that indirect transmission is more influential in determining infection patterns, but we do not rule out that direct transmission is possible in this system, or that it may affect severity of infection.

Dominance status and the risk of infection

One clear result of this study is that, in concert with other behaviors, the social hierarchy of house finches can affect an individual's risk of becoming infected. When we compared the top models that included dominance to equivalent models that excluded dominance, the dominance parameter always improved support. Model-averaged estimates strongly indicate that dominant individuals are less likely to become infected than subordinates. We again cannot precisely determine the

magnitude of this effect because the 95% confidence intervals overlap zero. Despite the consistent presence of dominance in the best-supported models, the fact that the dominance-only model performs poorly illustrates the complexity inherent in this system. This is likely why we did not see only individuals of low status becoming infected.

Dominance status has long been studied for its effects on immunocompetence and parasite susceptibility (Fairbanks & Hawley 2011). Among non-cooperatively breeding species, subordinates often have higher stress hormone levels (reviewed in Creel 2001), and Hawley (2006) showed a direct link between dominance and immunocompetence: house finches that were forced to become subordinate had a reduced immune response. In an experiment in which all house finches had been inoculated, Hawley et al. (2007b) found that dominant males (but not females) had lower disease severity and recovered more quickly. Our experiment extends our knowledge of the effects of dominance by showing that there is a close link between behavior and pathogen susceptibility. A possible explanation for increased susceptibility of subordinates could be differential resource access; however, there was no significant correlation between social status and feeder visitation rates, making resource access an unlikely explanation for our results. Given the correlational nature of these relationships and the fact that birds were fed *ad libitum* in captivity, the results should be interpreted with caution when considering patterns among wild house finches. However, these results do suggest that individual context-dependent behavior could have larger consequences for broad-scale disease dynamics.

Do diseased birds act as superspreaders?

Our results indicate moderate support for the role of index bird sociality as a driver of infection patterns, whereas the general sociality of susceptible individuals seems to have no effect on these dynamics. Our second best model included index sociality and dominance status, suggesting that the rate at which diseased birds socialize influences the likelihood that susceptible birds will become infected. However, this parameter is not a clear-cut predictor of infection risk. Alone or included in other transmission-only models, index sociality decreased model support; only in combination with dominance status did model support improve. Our ability to precisely estimate effects of index bird sociality was clearly weak, given that we were only able to conduct three replicates of this resource-intensive experiment. This prevents us from calculating the magnitude of the effect of sociality on disease risk, but we can say that there is some support for index sociality influencing infection risk among susceptible individuals. These patterns likely indicate that highly social diseased birds can at times increase the risk of infection for susceptible birds. For example, in aviary A, the index bird was both significantly more gregarious and had a higher dyadic interaction rate than did other index birds (Table 2.2). Although it was not significant, there were more qPCR positive birds in aviary A (Figure 2.1); furthermore, these birds were infected for a longer duration than in other aviaries.

These social individuals, once infected, may be spreading *M. gallisepticum* around the aviary. Previous work found that once an individual becomes infected, its behavior changes, increasing the opportunity for direct or indirect transmission: diseased house finches fed for significantly longer than individuals without lesions

(Hawley et al. 2007a). Furthermore, there is the opportunity for a positive feedback loop on infection: because of diseased birds' reduced aggressiveness, susceptible birds spend more time near them at feeders (Bouwman & Hawley 2010). Work in other systems found that highly social and infectious individuals disproportionately contribute to the spread of disease relative to less social individuals (Li et al. 2004; Lloyd-Smith et al. 2005). This further confirms the need to account for individual-based behavioral patterns in trying to understand disease dynamics, a field in which network models have been very useful (Bansal et al. 2007). Coupled with dominance status, index sociality plays a role, albeit a lesser role, in determining risk of *M. gallisepticum* infection in our system.

Conclusions

Taken together, these results indicate the importance of not simply a single transmission-relevant behavior, but rather a complex interplay of multiple behaviors that drive disease dynamics within a group. This work is also novel because it compares the importance of indirect and direct modes of transmission for a single pathogen; to our knowledge this has not been explored before.

There are some caveats in our work that limit extrapolation to wild house finch populations. Using qPCR as a measure of infection likely reflects both exposure and susceptibility, so it may be difficult to tease apart these two factors. We acknowledge that there can be sex-biased patterns of transmission in social organisms (Grear et al. 2009), including house finches (Hawley et al. 2007b; Bouwman and Hawley 2010). However, due to a limited collection of wild birds that caused an

uneven sex ratio, we were unable to examine the effects of birds' sexes in a conclusive manner. To have found sex-related differences in transmission risk would require a very strong effect of sex in our experiment (3 replicates with 11 birds each and an 8:3 ratio of females:males). In post-hoc analyses, the additional effect of sex consistently decreased the support of each model. Our groups of 11 birds may not adequately represent variation in social activity in the wild. However, we attempted to simulate wild conditions as much as possible: each bird had over 3 m^3 of space, which permitted individuals to avoid each other. Small sample sizes limited full interpretation of model-averaged estimates; however, the estimates were useful in that they provided an indication of the direction of effect on infection risk. Based on our construction of the four transmission model groups, we still see that some behaviors play a disproportionate role in patterns of infection. Furthermore, our base null model, representative of a homogenous population in which individuals exhibit no behavioral variation, has no support. Thus, while our work demonstrates the behavioral differences among individuals play important roles in allowing prediction of infection risk, the actual effects could not be estimated with high precision. In summary, we have shown the need to incorporate individual-level behavioral variation in order to more accurately predict population-level disease dynamics, with further studies needed to gain a more precise understanding of these effects.

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CHAPTER 3

ANTIMICROBIAL FEEDERS DO NOT REDUCE VIABILITY OF *MYCOPLASMA GALLISEPTICUM*

Abstract

Resource provisioning can have both positive and negative ecological and evolutionary consequences for wild populations. Resource sites or structures can serve as a mechanism of pathogen transmission, either by increasing intra-and interspecific aggregations, thereby facilitating contacts among infected and susceptible individuals, or as fomites. Because bird feeding is a common practice, feeders might influence pathogen transmission rates among bird species. In the house finch (*Carpodacus mexicanus*) – *Mycoplasma gallisepticum* host-pathogen system, feeders are known to be capable of transmitting the pathogen, but a clearer understanding of the relative importance of transmission modes is still needed in this system. A novel strain of the bacterium recently emerged in house finches, and causes conjunctivitis in the finches and other passerine species. A recently-released line of antimicrobial feeders potentially serve as a mechanism to test the relative importance of indirect transmission of *M. gallisepticum* through fomites. We inoculated EcoClean® and standard (non-antimicrobial) tube feeders with *M. gallisepticum* and sampled viability at regular intervals post-inoculation to determine whether the antimicrobial surfaces effectively reduce *M. gallisepticum* on the feeders. We find no significant differences between the feeder types. We also find wide variation in viability within and between sampling intervals, and these differences were significant in two out of the three

experiments. We conclude that even with some evidence for temporal declines in viability, the antimicrobial feeders do not seem to be an effective method to examine the relative importance of transmission mode in this host-pathogen system.

Introduction

Feeding wildlife is a common pastime in the United States and other countries (Davies et al. 2009). Over 71 million people (31% of the U.S. population 16 and older) observed, fed, or photographed wildlife in 2006 (USFWS 2006). Supplemental feeding has potentially large effects on wild species; while large-scale ecological effects are not well known, evidence suggests that supplemental feeding reduces the risk of starvation, and may enhance reproduction (Newton 1998) and timing of singing (Robb et al. 2008). Detrimental outcomes resulting from resource provisioning include such risks as dependence on human-provided foods, loss of foraging skills, changes in migration patterns, increases in interspecific and human-directed aggression, increased predation pressure, and pathogen spread. While not all of these risks have been substantiated (see review in Jones et al. 2008), other risks, such as pathogen transmission, are well documented (Dhondt et al. 2005, Benskin et al. 2009, other examples from Bradley and Altizer 2007).

Disease transmission at resource sites can be facilitated through a variety of mechanisms. Supplemental provisioning increases heterospecific contacts, which can increase the likelihood that healthy individuals contact infected individuals (Benskin et al. 2009). Provisioning may change community structure by increasing interspecific contact among species that might not otherwise interact (Jones and Reynolds

2008). Incidental feeding of non-target species can attract disease reservoirs that may contribute to pathogen transmission (Tompkins et al. 2001), though this is not always the case (Townsend et al. 2003).

The resource site or structure itself can be a fomite, an inanimate object capable of transmitting infectious organisms, and a variety of pathogens or toxins are known or suspected to be spread in this manner, including *Salmonella* (Daoust et al. 2000), *Trichomonas gallinae* (Anderson et al. 2009, Lawson et al. 2011), mycotoxins (Oberheu and Dabbert 2001), and *Mycoplasma gallisepticum* (Fischer et al. 1997). Previous work suggests that not only the density of individuals (Anderson and May 1978), but also the type of feeder contributes to pathogen transmission (Brittingham and Temple 1986, Hartup et al. 1998). Given the variety of bird feeders available, it is likely that feeder structures could influence contact rates and hence rates of transmission between susceptible and infected individuals. By extension, experiments in which different types of bird feeders are made available could be used to make inferences about the process of pathogen transmission.

In this regard, a recently-released line of bird feeders with antimicrobial surfaces (EcoClean®, Wild Birds Unlimited) are potentially useful in experiments designed to determine the importance of indirect transmission of pathogens via fomites. These feeders contain Agion®, a product impregnated with silver ions that have antimicrobial properties, and this material is found in the tube, coated metal components and perch covers of these feeders (Wild Birds Unlimited). Silver and silver ions have long been known to have antimicrobial effects (Gosheger et al. 2004). Implements coated with materials containing silver ions are increasingly used in

medical technology and have been documented to be effective in reducing or preventing a variety of infections (Simchi et al. 2011).

A host – disease system that is amenable to experimentation and for which use of antimicrobial feeders would be appropriate is house finches and the pathogen *Mycoplasma gallisepticum*. House finches are common, gregarious feeder birds, and visit multiple feeding sites within a local area, putting them in contact with different birds and feeders (Dhondt et al. 2007a). House finches and other songbird species are susceptible to the bacterium *M. gallisepticum*, which causes conjunctivitis in some species of passerine birds (Ley et al. 1996, Hartup et al. 2000). A well-characterized pathogen of domestic poultry, a novel strain of *M. gallisepticum* emerged in the winter of 1993-1994 and rapidly swept through house finch populations in the United States and Canada. The prevalence of mycoplasmal conjunctivitis is highest during the non-breeding season when house finches are most active at feeders (Hosseini et al. 2004). We know that transmission via fomites is possible: naive house finches can become infected through *M. gallisepticum*-contaminated feeders (Dhondt et al. 2007b). Still unclear is the relative importance of *M. gallisepticum* transmission via feeders and direct transmission of the pathogen among hosts. However, even in captive experiments it is challenging to independently manipulate these two modes of transmission to determine their influence on disease dynamics.

In this paper, we report on the efficacy of anti-bacterial bird feeders to kill *M. gallisepticum*. We conducted three experiments comparing viability of *M. gallisepticum* on antimicrobial EcoClean® tube feeders and standard tube feeders. We hypothesized that *M. gallisepticum* will be viable for a shorter time and

in smaller numbers on the antimicrobial feeders than on standard feeders. If this hypothesis is true, we can then use the antimicrobial feeders in future studies to better quantify and understand the role of transmission via feeders among house finch groups.

Methods

Experiment #1

We obtained 4 standard non-antimicrobial and 4 antimicrobial EcoClean® 6-port tube feeders (Wild Birds Unlimited, Inc.). All feeders were sterilized and placed approximately 0.5 m apart in an enclosed room (mean temperature = 16.8°C, mean humidity = 33.6%). One sterilized standard feeder used previously in *M. gallisepticum* studies served as a control for potential aerosol transmission (not inoculated, but surfaces sampled).

As in a previous experiment (Dhondt et al. 2007b), a 50 µl droplet of inoculum was applied on the lower (dependent) horizontal surface of each of the 48 feeder ports using a pipet and spread with a sterile plastic spatula. This quantity of inoculum is the standard amount used in similar captive infection experiments. Immediately prior to feeder application, we reserved a 50 µl sample of inoculum in 1ml Universal Transport Medium (UTM) (Copan Diagnostics Inc.) for a measure of viability. Inoculum consisted of the NC2006 strain of *M. gallisepticum* (accession 2006.080-5, ADRL NCSU CVM), the forth broth passage of an isolate from a diseased North Carolina house finch in 2006 (2006.080-5 4P, 1/9/09), with a viable count of 3.04 x 10^8 color changing units (CCU) per ml. This NC2006 inoculum has been used in

previous experimental infections and found to be highly pathogenic (Grodio et al. 2012).

Following application to the bird feeders, we sampled ports following an exponential time series at 0.5, 1, 2, 4, 8, 16 hours on 21 October 2011. To establish that no *M. gallisepticum* was present prior to inoculation, approximately 24 hours prior to application of *M. gallisepticum* to the feeder surfaces, we swabbed the feeders to collect "time -24hrs" viability samples. At each time interval, a sample was taken from one feeder port on each feeder with the feeder port randomly selected in advance, so that a different feeder port was sampled at each sampling time. The port was swabbed with one wetted swab and swirled in 1ml UTM. Feeders were sampled in a different random order at each time interval. Swabs of each port (n=6) were pooled for each feeder (n=9) for a total of 9 samples for *M. gallisepticum* culture.

Following collection and frozen storage at -70°C of all samples they were shipped without thawing on dry ice to the Mycoplasma Diagnostic and Research Laboratory (NCSU CVM, Raleigh, NC, USA) for viability testing. Upon arrival, samples were thawed, 200 ul of each were added to Frey's broth medium with 15% swine serum, and incubated in humidified air at 37°C. If growth occurred viability counts were made from selected time intervals of antimicrobial and standard feeders.

Viable Count Analysis

To determine the abundance of viable *M. gallisepticum* in a sample, color-changing units (CCU/ml) in a microtitre format were calculated by the most-probable number (MPN) method (Meynell and Meynell, 1970).

We found that 3 of 8 of our "-24 hours" samples were contaminated with fungal growth. We reran the viability counts using FMS/Fungizone for these samples and hour 16 samples; no fungal growth was found. To prevent fungal contamination, we used Fungizone in all subsequent viability counts.

Experiment #2

Because *M. gallisepticum* has no cell wall and limited metabolic capability, in nature it is vulnerable to environmental insults and does not replicate or maintain viability for long outside of a suitable host (Razin 1995). Due to fungal contamination which may have contributed to unexpected temporal variation in viable counts in experiment #1, we wanted to examine *M. gallisepticum* viability over time on a neutral surface and use media with Fungizone. We marked 2 2-cm circles on 14 glass slides, and each slide was placed in its own sterile Petri dish and autoclaved. We used the NC2006 *M. gallisepticum* strain and passage identical to that used in Experiment #1 (4th in vitro passage, 2006.080-5, 01/09/2009).

We inoculated slides by placing a 20 µl drop of inoculum within each circle on each slide and spread each drop to cover the marked area. The Petri dishes were covered and the slides allowed to dry. Slides within Petri dishes were kept at room temperature and humidity in an air-conditioned laboratory without exposure to sunlight. We performed a 'positive control' viable count on 250 µl inoculum using FMS/Fungizone broth and incubated at 37°C for 2 weeks.

On each day in the period from 1 to 7 days post-inoculation, we placed a 20 µl drop of sterile FMS/Fungizone broth on each of the 2 marked, previously inoculated

areas on each of 2 slides. The drop was spread over the entire marked area, and then swabbed with a dry swab and transferred to a tube containing 1 ml sterile FMS/Fungizone broth (4 total samples per sampling day). We then performed growth/no growth and viable cell counts for each of the samples. Viable counts were determined by the color changing units (CCU) method, a statistical approximation of viability based on serial dilutions in a microtiter system. These were incubated at 37°C for 2 weeks.

Experiment #3

Because our neutral surface experiment showed a decline in *M. gallisepticum* viability over time, we repeated our comparison of viability on standard and antimicrobial feeders (Experiment 1).

To ensure that we evenly and accurately applied the inoculum to the feeder port surfaces, we removed all painted metal ports from 4 standard and 4 antimicrobial 6-port feeders. These were new feeders and were cleaned in soap and water and rinsed thoroughly with de-ionized water prior to the start of the experiment. All ports were placed on a stable, sterilized surface. We used the same NC2006 inoculum as was used in both previous experiments (4th in vitro passage, 2006.080-5, 01/09/2009). We sampled ports daily for 5 days, and included a '0' time interval which we sampled approximately 35 minutes after inoculation.

We placed a 20 µl drop of inoculum in the center of the designated area on each port and spread the drop with a pipette tip to cover an area approximately 2 cm in diameter. To sample the ports, we placed a 20 µl drop of FMS/Fungizone broth on the

inoculated area of 6 randomly-selected ports (3 antimicrobial, 3 standard) that had not previously been sampled at each time interval. We swabbed the area with a dry swab, and was transferred it to a 1 ml tube of sterile FMS/Fungizone broth. We again performed viable counts on each sample in microtitre plates, incubated at 37°C for 2 weeks, and checked for evidence of color change daily. The rest of the samples were incubated at 37°C for growth/no growth determination for 3 weeks.

Data analysis

We used a repeated measures ANOVA to model changes in viability through time, and in Experiments 1 and 3, to compare viability between standard and antimicrobial feeders. A repeated measures ANOVA allowed us to compare changes in the dependent variable on the same subjects when measurements of each subject were made repeatedly. Because our viability counts were overdispersed, we used log-transformed values to meet normality assumptions of ANOVA. All analyses were conducted using R statistical software (see http://www.r-project.org).

Results

Experiment #1

All feeders, including the control (non-inoculated) feeder, tested negative for *M. gallisepticum* at the "-24 hours" time period. We ran viability counts on swabs from 2 of the 4 feeders in each treatment group for the 0.5, 1, 4, and 8 hour sampling periods, and on none from the 2-hour period. Viability counts were first run on the later sampling periods, and because we observed no significant differences between

treatments (see below), viable counts from the 2-hour period were unnecessary. Viable counts were made on all 8 samples from hour 16. Our undiluted viable count of the inoculum was 4.6 x 10^5 CCU/ml, which verified that *M. gallisepticum* was present in the inoculum.

Our repeated measures ANOVA indicated no significant differences between antimicrobial and standard feeder viability counts (Figure 1; ANOVA: $F_{1,4} = 4.54$, P = 0.10). We also found that neither group showed significant changes in viability over time (ANOVA: $F_{4,8} = 1.62$, P = 0.26).

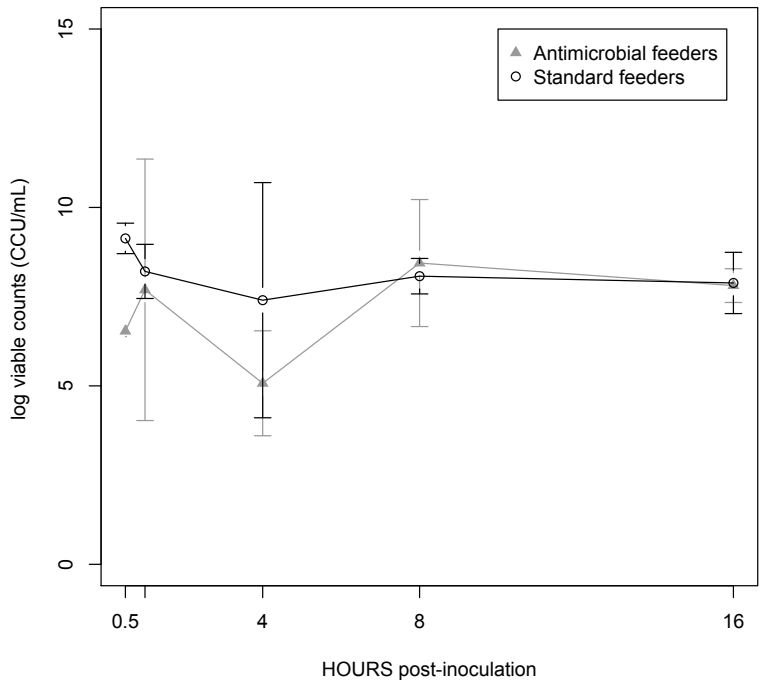

Figure 3.1. Viable counts (CCU/ml) on antimicrobial (triangle symbols) and standard (circle symbols) feeders. Sampling was performed over an exponential time series, from 0.5 to 16 hours post-inoculation.

Experiment #2

In this experiment, the undiluted inoculum viable count was 1.15 x 10^8 CCU/ml. Viability showed a steady temporal decline on the slides, and viability was 0 on day 7 (Figure 3.2). Our ANOVA indicated a strongly significant effect of time on viability (ANOVA: $F_{6,18} = 64.98$, $P < 0.001$).

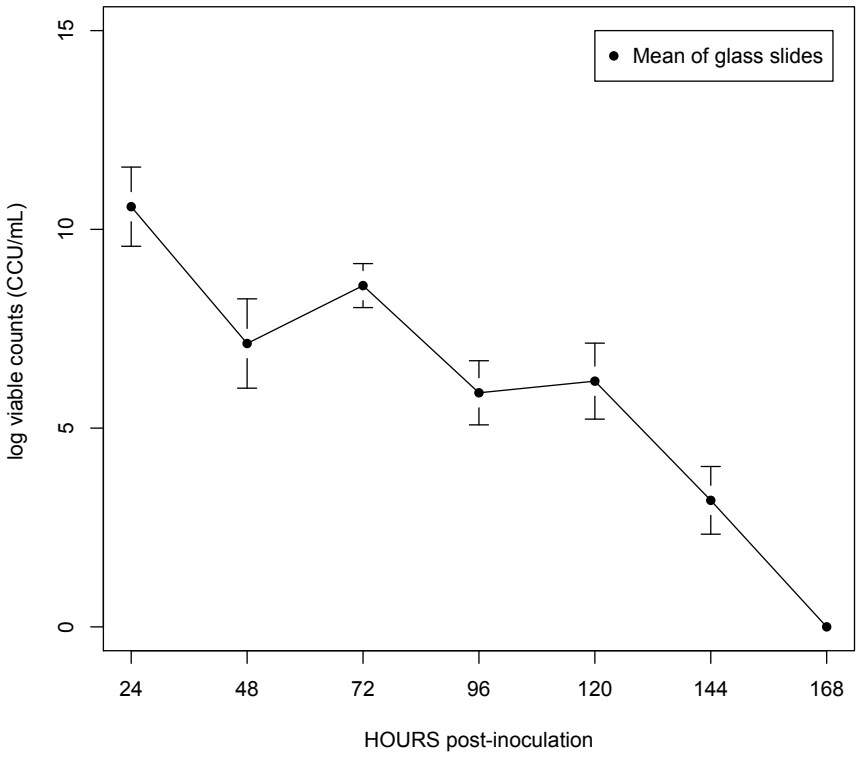

Figure 3.2. Viable counts (CCU/ml) on glass slides sampled every 24 hours for 7 days. Glass slides represented a neutral surface on which to observe changes in viability over time.

Experiment #3

We ran viable counts on 6 feeder ports for each time interval (3 antimicrobial, 3 standard). The count of viable *M. gallisepticum* from the inoculum at time 0 was 4.6 x 10^6; the 1:50 diluted inoculum viable count was 6.36 x 10^4 CCU/ml. There was no significant difference in viability counts between treated and untreated feeders (Figure 3.3; ANOVA: $F_{1,1} = 60.857$, $P = 0.08$). Both treatments showed near-parallel fluctuations in viability counts across the sampling periods, though overall they showed significant changes in viability over time (ANOVA: $F_{1,5} = 23.6115$, $P < 0.001$).

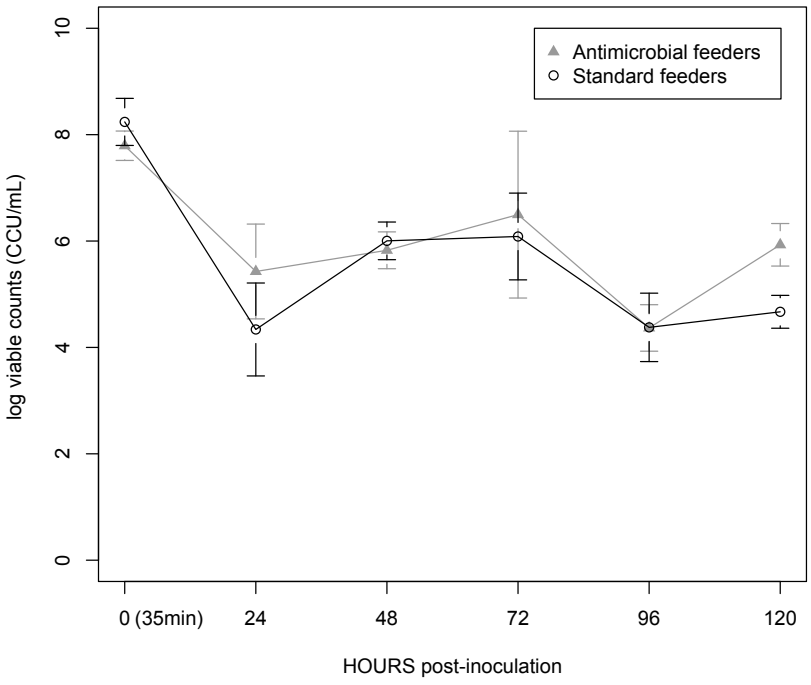

Figure 3.3. Viable counts (CCU/ml) on antimicrobial (triangle symbols) and standard (circle symbols) feeders. Samples taken at 35 minutes post-inoculation and then every 24 hours for 5 days.

Discussion

The ability to reduce or eliminate indirect pathogen transmission via fomites would provide an easy and novel method for comparing transmission modes in an experimental setting. In our comparisons of antimicrobial and standard feeders, we found no significant differences in *M. gallisepticum* viability. In both Experiments 1 and 3, despite nearly identical sampling techniques and conditions, and with the same *M. gallisepticum* inoculum, we found no evidence to suggest that antimicrobial feeders reduced the abundance of viable bacteria on the feeder.

Failing to find differences could be due to several factors. Agion® materials may not effectively kill *M. gallisepticum* cells. Agion® states that the silver ions use three methods to reduce microbes: 1) they prevent respiration by inhibiting transport functions in the cell wall, 2) they inhibit cell division, and 3) they disrupt cell metabolism. Like all mycoplasmas, *M. gallisepticum* does not have a cell wall (Razin 1995), nor can it reproduce outside of its host, so Agion® materials would not be effective through the first two methods. We do not know how silver ions might affect cell division and metabolism in *M. gallisepticum*, so we cannot speak to the efficacy of these mechanisms. It may be that Agion® antimicrobial activity requires a nutrient environment that supports growth and replication, which was only present for the short time that it took for the inoculum to dry on the port surface.

Independent analyses of the Agion® product found no effect of treatment: both control and treated samples showed a greater than 99% reduction in bacteria viability after 24 hours (WBU Inc., personal communication). Those results and ours suggest that there is no confirmation that the Agion® antimicrobial surface reduces viability of

M. gallisepticum. However, similar independent tests of other bacteria, such as *Salmonella* spp., did show > 99.9% reductions of bacterial presence on antimicrobial surfaces (WBU Inc., personal communication).

Another possible reason that we did not find a difference between feeder types is that the efficacy of the silver ions may have been affected by our experimental conditions. This could have been due to variation in both inoculation of the feeders and sampling of the dried inoculum, and the high dosage of *M. gallisepticum* applied to the feeders. The curved surfaces of the feeder ports may have caused the inoculum to pool, creating a thicker layer that could have reduced effectiveness of the silver ions. Variation in swabbing pressure or surface coverage when sampling could affect results. We also might have disproportionately sampled the top layer of inoculum that could have had less exposure to the silver ions. Finally, we did not test if the bacteria load present in the undiluted inoculum is comparable to loads present in infected wild passerines. If the inoculum dose was exceptionally high, it may have been that the antimicrobial materials could not work effectively under such a high dose of the bacterium.

We found that there was a significant decline in *M. gallisepticum* viability counts through time in our second and third experiments, and much variation in viability within weeks. These results fit our prediction of a temporal decline in *M. gallisepticum* viability, since it cannot survive or reproduce outside its host. However, variability in viable counts among weeks may also be a result of sampling inconsistencies, or variation in viable count analyses.

These three experiments provide new evidence of somewhat longer-than-

expected survival of *M. gallisepticum* outside the host. Our environmental conditions post-application were very benign: laboratory room temp and humidity, with no exposure to sunlight; real life outdoor conditions would likely yield different results. Previous work found that *M. gallisepticum*-inoculated feeders did not transmit the bacterium to naive house finches 24 hours after inoculation (Dhondt et al. 2007b). In experiments 2 and 3 we found that some bacteria were viable for up to 6 days post inoculation.

Although the antimicrobial feeders do not appear to be a useful method to parse out the effects of transmission mode on disease dynamics, these results challenge our previous assumptions of the length of pathogen viability, and suggest that fomites may contribute even more than previously thought to disease dynamics in the wild. Further examination of *M. gallisepticum* viability and load required to constitute an infectious dose would be crucial to our understanding of both the contribution of fomites and pathogen load to disease dynamics.

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CHAPTER 4

FACTORS DRIVING INFECTION RATES IN HOUSE FINCHES

Abstract

While characteristics of both a pathogen lineage and an individual host will affect the probability of a new host becoming infected, the ecological context — the characteristics of the host population as a unit — can also play an important role in determining the rate of pathogen transmission to new hosts, and ultimately affect the outcomes of disease epidemics. We experimentally evaluate the relative importance of several biotic and abiotic factors that may affect the rate at which a pathogen will spread through a population. Specifically, we examine the relative importance of pathogen abundance within and distribution among infectious hosts and potential interactions with host sex and resource use. We use multi-state mark-recapture models to examine how these factors affect the rates at which individual house finches (*Carpodacus mexicanus*) will become infected with the bacterium *Mycoplasma gallisepticum*, in a series of flocks housed in large, semi-outdoor, free flight aviaries. Our results indicate that pathogen-related characteristics can drive host infection patterns: the total pathogen load and the distribution of the pathogen among a group of hosts influences the probability that a host individual becomes infected. We also find that the sex ratio of a host population will affect rates of transmission, because male house finches have higher infection rates than females. These results suggest that among-host variation in pathogen distribution, which can vary through time, is an

important driver of transmission patterns within a host population.

Introduction

Pathogen transmission is a key epidemiological process and identifying the causes of variation in transmission rates is vital to understanding population-level host – pathogen relationships and the outcomes of a disease epidemic. At a population level, transmission is typically explained as an effect of the mass-action principle, wherein transmission depends on both the number and rates of contact between susceptible and infected hosts present in the system (Heesterbeek et al. 1995). However, populations of hosts are not homogenous, nor are pathogens equality distributed among hosts, which can cause variation in transmission rates in different host populations (Grundler et al. 2012). Biotic factors such as differential susceptibility to the pathogen (Wilson et. Al. 2002, Beldomenico and Begon 2002) or behavioral or physiological differences among hosts (Klein 2004; Hawley and Altizer 2010), and abiotic factors such as seasonality and differential resource access (Altizer et al. 2006; Robb et al. 2008; Benskin et al. 2009) may all cause variation in the distribution and abundance of a pathogen within a host population. While this in turn can lead to differences in transmission rates of the pathogen, we still need to more clearly elucidate the relative importance of how these host- and pathogen-specific factors contribute to variation in the rate that a pathogen spreads within a group of hosts.

Characteristics intrinsic to a pathogen can affect the distribution of the pathogen within a host population. Replication rate and virulence, traits modulated in

part by the pathogen, affect its distribution and abundance among hosts, and can thus drive variation in infection outcomes within these hosts (Ben-Ami et al. 2008; de Roode et al. 2008). This variation can alter not just the number of infected individuals, but also both the total amount of pathogen, or "load," and how evenly the load is distributed among hosts. Pathogen load tends to have a skewed distribution in a host population, where a few hosts have a high pathogen load, but most have a low-level or nonexistent infection (Bertolino et al. 2003).

Host-specific factors can also drive infection patterns, or interact with pathogen-specific characteristics. The sex of the host has long been known to affect patterns of susceptibility to, and recovery from, infection, but this relationship is complex and lacks consistency (Zuk and McKean 1996; Ardia et al. 2011; Lachish et al. 2011). There can also be sex-specific patterns of interaction among hosts, often hormonally mediated, which can subsequently influence transmission rates (Fairbanks and Hawley 2011) in host populations with different sex ratios.

Further, resource distribution may affect rates of contact among hosts, and in this way indirectly affect transmission rates; this has been seen for resources such as territory or shelter (Godfrey et al. 2009, Lindner et al. 2011) as well as food (Jones et al. 2008). Beyond influencing host interaction, the resource sites themselves can often harbor, and thus distribute, pathogens (Fischer et al. 1997, Anderson et al. 2009). In the case of wild bird populations, aggregations at supplemental food, and sometimes the type of feeding structure, are suspected to determine the rate of pathogen transmission (Brittingham and Temple 1986, Hartup et al. 1998).

House finches (*Carpodacus mexicanus*) and the bacterial pathogen

Mycoplasma gallisepticum are an ideal system in which to examine how ecological variation influences group infection dynamics. A common infectious agent in domestic poultry, *M. gallisepticum* emerged in house finches in the winter of 1993-94 and rapidly spread across the United States and Canada (Ley et al. 1996, Dhondt et al. 1998). The bacterium causes conjunctivitis in house finches, but is found in, and can be transmitted by, other songbird species (Fischer et al. 1997, Dhondt et al. 2008). The initial outbreak of *M. gallisepticum* in house finches reduced populations in a density-dependent manner; there were declines of up to 60% in some areas (Hochachka and Dhondt 2000).

Previous work has explored the effects of host- and pathogen-specific characteristics on transmission of *M. gallisepticum* within house finch populations. We know that sex-based differences in infection are complicated in this system: dominant males had lower disease severity and recovered more quickly from infection (Hawley et al. 2007); however, other previous work did not detect an effect of sex, possibly due to small sample sizes (Chapter 1). Still, sex ratio differences might have the potential to influence population-level transmission patterns. We also predict that resource provisioning might structure disease dynamics, given that 1) house finches are gregarious and common at feeders, 2) early field data documented greater incidences of diseased house finches at sites with tube feeders (Hartup et al. 2000), and 3) experimentally infected house finches can contaminate feeders, and naive individuals can be infected when exposed to these infected feeders (Dhondt et al. 2007). Only recently have we begun to explore the importance of pathogen variation; for example, recent work has shown that differences in average pathogen prevalence,

linked to genetic differences among *M. gallisepticum* strains, will affect population-level patterns of infection (Hawley et al. 2010). However, we still know little about how variation in pathogen load and distribution may drive infection dynamics within a population.

In this paper, we ask how variation in several biotic and abiotic factors may force infection probabilities within susceptible individuals, using temporal changes in these factors to identify important causes of variation in rates of host infection. Multi-state mark-recapture models provide a novel method to measure weekly variation in the rates at which susceptible hosts become infected by *M. gallisepticum* in captive groups of house finches. By quantifying these factors and monitoring disease dynamics, we have estimated individual infection probabilities from week to week and determined which factors most strongly influenced the course of infection within groups of house finches.

Methods

Experimental Setup

Juvenile house finches were caught using mist nets or wire mesh traps in Ithaca, New York, USA (42° 51'N, 76° 34'W) in late summer and fall 2010 under USGS Bird Banding Lab permit #23513. After capture, birds were banded with colored leg bands for identification and housed individually for a minimum quarantine period of 2 weeks. Plastic dividers between all cages prevented interaction and potential indirect transmission during this period. During quarantine, birds were tested for presence of *M. gallisepticum* by quantitative PCR (qPCR) analysis (Grodio et al.

2008), for *M. gallisepticum* antibodies using enzyme-linked immunosorbent assay (ELISA) (Grodio et al. 2011), and inspected for the presence of eye lesions. Birds were tested within 2 days of capture, and again 7 days prior to the beginning of the study. Only birds that were negative for the bacterium in all tests were used in the experiment. We determined sex of the birds with a molecular-based PCR assay to amplify the sex-specific CHD-W and CHD-Z genes as in Griffiths et al. (1998).

We randomly assigned 64 hatch-year individuals (34 females, 30 males) to 6 separate flocks; sex ratios remained approximately equal in each. Each flock (10 or 11 birds) was housed in a large, free flight, semi-outdoor aviary. Each aviary was a peaked octagonal room, 2.9 m in diameter and ranged from 2.4 m to 3 m high. Three aviaries were designated as platform feeder treatments, 3 as tube feeder treatments. Food was provided in either a clear plastic tube feeder with 6 openings (ports) or on a 30.5 cm by 30.5 cm wooden hanging platform feeder. There was 1 feeder and 1 water dish per aviary. Water and pelleted food (Roudybush, Inc., Cameron Park, CA, USA) were provided *ad libitum*. Artificial trees and wreaths were placed in the aviaries to provide perches and cover and were arranged identically among aviaries. Heating was provided near perches and water dishes using infrared lamps.

Measurements of Infection

On 3 February 2011, one bird from each flock was selected to be the index host for *M. gallisepticum* transmission. To minimize inter-cage variation based on behavioral differences of the index birds, we chose birds that had moderate levels of interaction with other individuals and the feeder, and were in the middle of the

dominance hierarchy. Birds were observed for three weeks prior to inoculation following behavioral observation methods in Chapter 2. These birds, 3 females and 3 males, were inoculated bilaterally in the conjunctiva with 0.05 ml of *M. gallisepticum* stock inoculum (4th in vitro passage of the original house finch *M. gallisepticum* isolate 2006.080-5). Index birds were held individually in a paper bag for at least five minutes to ensure absorption of the inoculum, after which these birds were returned to their aviaries. These birds were the initial source of pathogen that could be transmitted to other susceptible birds in each aviary.

To monitor infection status, every bird was trapped at day 6, 12, and 18, and then weekly for 9 weeks until 25 April 2011. At each capture, we quantified disease severity by scoring physical symptoms in both eyes on a 0–3 descriptive scale (see Sydenstricker et al. 2005). We also swabbed both conjunctivae to test for presence of the bacterium by qPCR analysis (Grodio et al. 2008). Each eye swab was analyzed separately. One week after inoculation and biweekly after that we took blood samples by venipuncture to test for *M. gallisepticum* antibodies using ELISA (Grodio et al. 2011). All animal handling and maintenance procedures were approved by the Cornell University Institutional Animal Care and Use Committee (#2006-094).

Predictor variables

Our primary goal was to determine the relative influence of sex, feeder type and the group measures of pathogen abundance and distribution on the initial transition of birds from the susceptible to infected state. Sex (*sex*) and feeder type (*feeder*) were treated as dichotomous variables. Our three pathogen distribution

measures were weekly-varying continuous covariates representing the total pathogen levels within the cage in which an individual bird resided. These three measures were: 1) the total number of infected individuals within the group (*numinf*), 2) the total load of *M. gallisepticum* within the group (*load*), and 3) the evenness of pathogen distribution within the group (*even*). These measures included the index bird when it tested positive, as these individuals were likely to affect pathogen dynamics within their respective groups. The total number of infected individuals was calculated as the sum of all individuals that tested qPCR positive in a given week. The total *M. gallisepticum* load was calculated as the sum of qPCR loads across both eyes for all qPCR positive birds in a given week. We did not assume that transition rate would increase linearly with total pathogen load because it is possible that the effect of load will asymptote. Hence in our analyses we used the base-10 logarithm of total load as our predictor variable. Preliminary analyses found this biological intuition to be valid: log-transformed load (*log(load)*) fit the data far better than the untransformed measure of total pathogen load.

Our measure of pathogen evenness was derived from Simpson's diversity index

$$D = \sum p_i^2$$

where p_i represents the proportion of individuals in the *i*th species (Simpson, 1949). In the context of pathogen distribution within a population, p_i is each infected individual's total pathogen load as a proportion of the group's total pathogen load. We used Simpson's diversity index rather than Simpson's measure of evenness, which standardizes the value by the total number of species in the sample (reviewed in

Magurran 2004) to make the measure of evenness independent of the total number of individuals. This standardization would not be appropriate in our system, because we want the distribution of the pathogen across all possible individuals in the group and not merely the number of pathogen-bearing individuals. Our preliminary analyses indicated no effect of aviaries (as a categorical predictor) on transition probabilities. Hence, we assume that any among-aviary variation in disease dynamics is largely or entirely due to variation in the biologically-relevant predictors that we measured, and not the result of differences in unmeasured characteristics of the aviaries.

Data Analysis

We analyzed our data using a multi-state mark-recapture approach, in which individuals in a population are treated as being distributed across multiple sites or states (Williams et al. 2002). These models allow a robust estimation of transition probabilities among states even when the probability of observing an individual at a particular sampling occasion is <1. Though typically used for wild populations, multi-state mark-recapture models can be used for captive populations as well, where survival and encounter rate probabilities are 1. Multi-state models typically assume a first-order Markov process, where the probability of a bird transitioning between disease states from time i to $i+1$ is dependent on its state at time i. Multi-state mark-recapture models are useful for wildlife disease studies, where individuals can be distributed among and move between disease states (Cooch et al. 2012). In our study, individuals were either susceptible (S) or infected (I), based on presence of *M. gallisepticum*, and transitions between disease states were interpreted as probabilities

of infection or recovery. These models assume certainty of classification of an individual within a particular disease state, an assumption we believe that our data met because 1) we were able to sample every bird in a consistent manner at each sampling interval, 2) no correlations existed between detectable measures of infection and host characteristics (i.e. sex, behavior), so we did not expect to find these patterns with infections that might have circulated below our level of detection, 3) our diagnostic methods use strongly validated qPCR techniques, which provide the earliest possible and most sensitive form of detection of the bacteria (see Grodio et al. 2008). These models are also useful to us because the transition probabilities can be functions of both constant and time-varying covariates.

Our mark-recapture data consisted of weekly individual measures of infection over 12 sampling periods. For these models we determined the initial week of infection as being the week of the first qPCR positive conjunctival sample for an individual, because this is the earliest indicator of infection. All individuals were grouped by sex and feeder type and initially were assigned to the susceptible (S) disease state. All state transitions were possible, as infected birds could recover, and could also become infected again before termination of the experiment; however, we only report on transition rates between susceptible and initial infection so that our models reflect our specific question regarding how these factors determine the initial transition from S to I.

All models were fitted to the data using program MARK (White and Burnham, 1999). Selection among models in the candidate model set was based on comparisons of the Akaike Information Criteria corrected for small sample sizes (AIC_c) (Lebreton

et al. 1992, Burham and Anderson 2002). AIC_c allows selection of the best approximating model for the data, based on principles of parsimony and trade-offs between under- and over-fitting models (Burnham and Anderson 2002). The best model among the candidate set of models had the lowest AIC_c value, and other models were ranked relative to deviations from the best model (ΔAIC_c). We also calculated cumulative AIC_c weights of models within the candidate model set to evaluate parameter importance (Burnham and Anderson 2002).

Our general model for transition rate was time-varying; we asked if the likelihood of becoming infected was best predicted simply by the underlying weekly temporal variation. We then developed an *a priori* candidate set of models in which transition rates were predicted to be additive effects of combinations of all covariates. We also included interactive effects of sex and feeder type and with each of the pathogen distribution measures, but did not include interactions of pathogen distribution parameters due to their lack of biological interpretability.

We then used Markov chain models to extend our exploration of the sex differences in infection rates. These models are useful for understanding likelihoods of infection and recovery in a disease epidemic by scaling up individual-level estimates to make inferences about population-level infection dynamics. We modified the Zipkin et al. (2010) Markov chain models to our captive system where survival and encounter rates of individuals are 1 to explore how differential transition probabilities in males and females might cause differences in both the probability of initial infection and the cumulative probability of becoming infected. We use the equation

$$f_{01}^{(m)} = \Pr\{x_{n+m} = 1, X_{n+m-1}, \ldots, X_{n+1} = 0 | X_n = 0\} = p_{00}^{m-1} p_{01}$$

to calculate the probability that a susceptible individual becomes infected for the first time between the m-1 and m time steps. This model assumes constant transition rates over time, so the simplification to $p_{00}^{m-1} p_{01}$could not be used in our estimation of cumulative infection rates. We then used the probabilities of first transitions to determine the cumulative probabilities that a susceptible individual became infected.

Results

Health status results for index and susceptible birds

Of our index birds, only 5 of the 6 became infected; the sixth bird, in a tube feeder aviary, never tested qPCR positive for *M. gallisepticum* nor developed conjunctivitis. There was no epidemic of mycoplasmal conjunctivitis in this aviary, so data from this aviary were not included in our analysis. All remaining index birds were qPCR postive at 6 days post-infection (hereafter, PI), and were positive for an average of 7.8 weeks.

A total of 43 susceptible individuals became infected; 6 other susceptible individuals never tested qPCR positive for infection. These birds that were negative for *M. gallisepticum* infection were evenly distributed among the aviaries. This pattern of failure of all susceptible individuals to become infected is typical in our experiments (Sydenstricker et al. 2005, Dhondt et al. 2007). Secondarily infected birds became qPCR positive at many different points during the experiment; times to initial infection ranged from 1 to 11 weeks PI, with an average of 4.12 weeks.

Though there was no significant difference in the final proportion of infected

birds based on feeder type (Fisher's exact test: p-value=1), the rate at which susceptible birds became infected was higher in platform feeders (Figure 4.1). We found no difference in the duration of infection based on feeder type; tube feeders resulted in a mean duration of infection of 5.35 weeks, platform feeders for 5.34 weeks (Welch's Two Sample t-test: t= 0.005, df=40.775, P = 0.99)

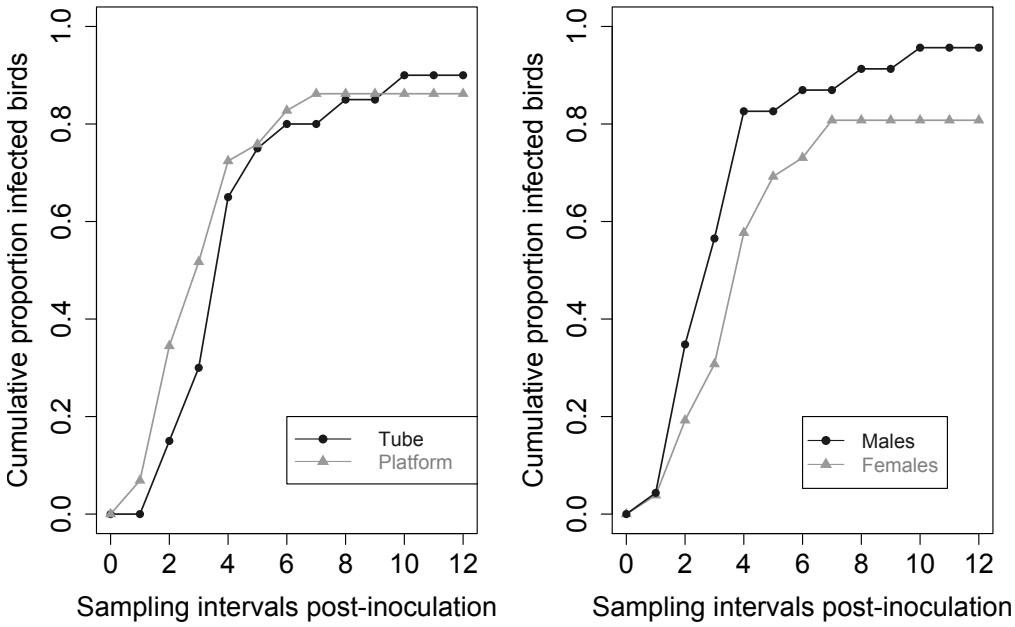

Fig. 4.1. The cumulative proportion of house finches secondarily infected with *M. gallisepticum* over the course of our experiment. On the left, the black circles represent birds in aviaries with tube feeders, grey triangles represent birds in aviaries with platform feeders. On the right, black circles represent males, grey triangles represent females.

There was no difference in the overall proportion of infected individuals by sex (Fisher's Exact test: P = 0.19); however, susceptible males became infected at a faster

rate than susceptible females (Figure 4.1). Among males and females, there was no significant difference in the duration of infection; females were infected for 4.81 weeks, males for 5.96 weeks (Welch's Two Sample t-test: t=-1.21, df=46.93, P = 0.23).

Despite the lack of overall mean differences, *a priori* we expected that there could be weekly differences in the likelihood that individuals became infected based on sex, the type of feeder from which they fed, or the weekly changes in group pathogen dynamics. Because both infection status and covariates vary throughout the course of the experiment, we employed a multi-state mark-recapture approach because it particularly useful for addressing the contribution of time-varying covariates to infection rates.

Multi-state mark recapture results

We found that that the consequences of infection dynamics depend on some, but not all of time-varying group covariates. There was strong support for models in which transition rate was a function of pathogen load and evenness (Table 4.1). The top 5 models include additive or single effects of these parameters, suggesting that they contribute most to transition rates. Models 6 and 7 both have ΔAICc <2, suggesting they have some support; both contained the evenness and load parameters, in addition to containing interactions among predictors. Both parameters also had high cumulative weights; load and evenness received cumulative weights of 0.722 and 0.216, respectively. Parameter estimates indicated that higher pathogen loads and a more uneven distribution of *M. gallisepticum* (meaning that the pathogen is

concentrated in a few hosts) lead to higher transition rates. These models also indicate

Table 4.1. Summary of multi-state mark-recapture analysis modeling infection rates of house finches by *Mycoplasma gallisepticum.* Transitions of house finches from susceptible to infected states were modeled as dependent on sex (*sex*), feeder type (*feeder*), and temporally-varying characteristics of pathogen distribution and abundance. These characteristics were the number of infected hosts (*numinf*), pathogen abundance in the hosts (*log(load)*), and the evenness of pathogen distribution among hosts (*even*).

#	Model	*K*	AICc	ΔAIC_c	w_i
1	sex + log(load) + even	5	571.091	0	0.253
2	sex + log(load)	4	571.776	0.686	0.180
3	log(load)	3	572.281	1.190	0.140
4	even	3	573.377	2.286	0.081
5	sex + even	4	573.440	2.349	0.078
6	sex * log(load)	5	573.770	2.680	0.066
7	sex * even	5	573.810	2.719	0.065
8	feeder + log(load)	4	574.121	3.031	0.056
9	feeder + even	4	575.372	4.281	0.030
10	feeder * log(load)	5	575.486	4.395	0.028
11	feeder * even	5	577.397	6.306	0.011
12	time	13	577.558	6.467	0.010
13	sex	3	582.255	11.164	0.001
14	sex * feeder	5	583.078	11.988	0.001
15	sex + numinf	4	583.427	12.336	0.001
16	sex + feeder	4	584.281	13.190	0.000
17	feeder	3	584.375	13.284	0.000
18	sex * numinf	5	584.549	13.459	0.000
19	feeder + numinf	4	585.754	14.663	0.000
20	feeder * numinf	5	587.138	16.047	0.000

that the number of infected individuals has no effect on transmission dynamics; indeed, the ΔAIC of any model that included this parameter was > 12.

In determining probabilities of transmission, models including feeder type had little support (ΔAICc > 3), even when important group covariates of pathogen load and evenness were included. Model-averaging demonstrated the lack of a difference between transition rates in tube and platform feeder treatments. Model-averaged estimates generated for feeder types were almost identical across the feeder types, and confidence intervals overlapped at all time intervals (Figure 4.2).

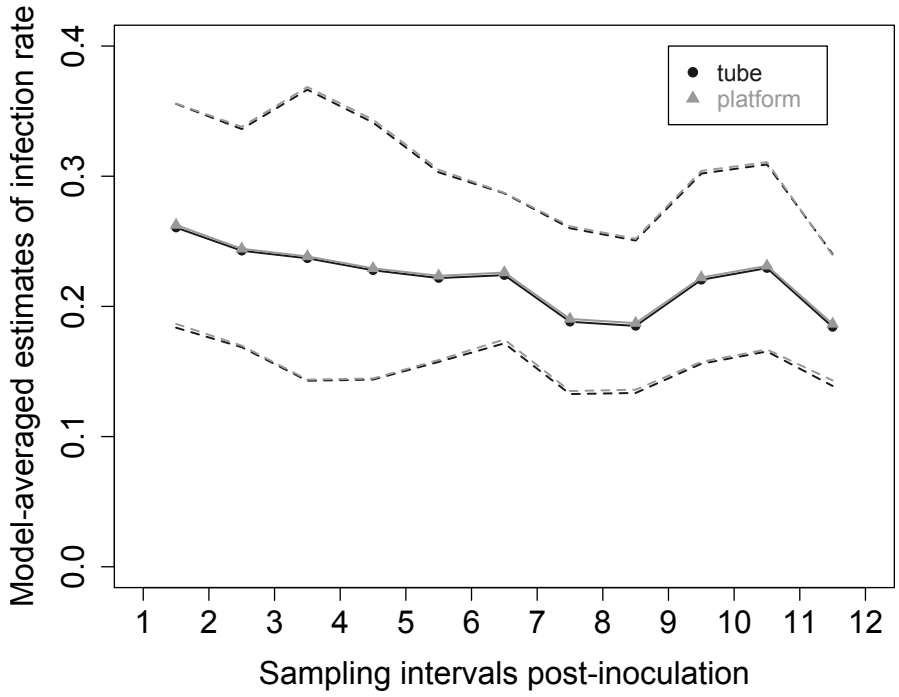

Fig. 4.2. Model-averaged estimates (+- 95% CI) from the multi-state mark-recapture analysis showing transition rates from susceptible to infected state of house finches. Black circles represent tube feeders, grey triangles represent platform feeders.

We also found evidence for sex-based differences in infection rates, as the top 7 models all included an effect of sex. Model-averaging of transition rates based on sex yielded consistently higher transition rates for males than females (Figure 4.3), indicating that males are more likely to become infected than females. Our models provide strong support for sex differences in transition rates, and despite the large confidence intervals in our model-averaged estimates, they suggest that differences in sex ratio might affect disease outcomes in a population.

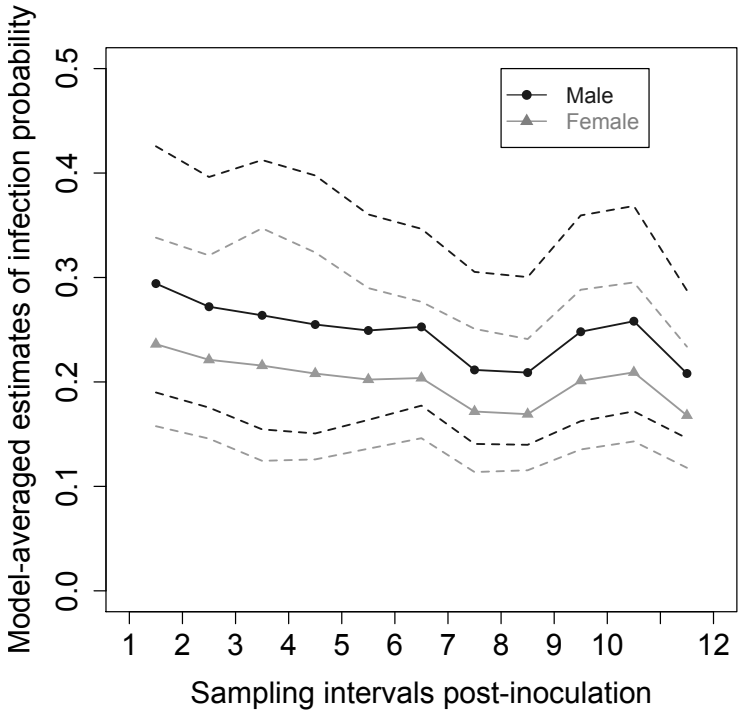

Fig. 4.3. Model-averaged estimates (+- 95% CI) from the multi-state mark-recapture analysis showing transition rates from susceptible to infected state of house finches over the duration of the study. Males are represented by black circles, females by grey triangles.

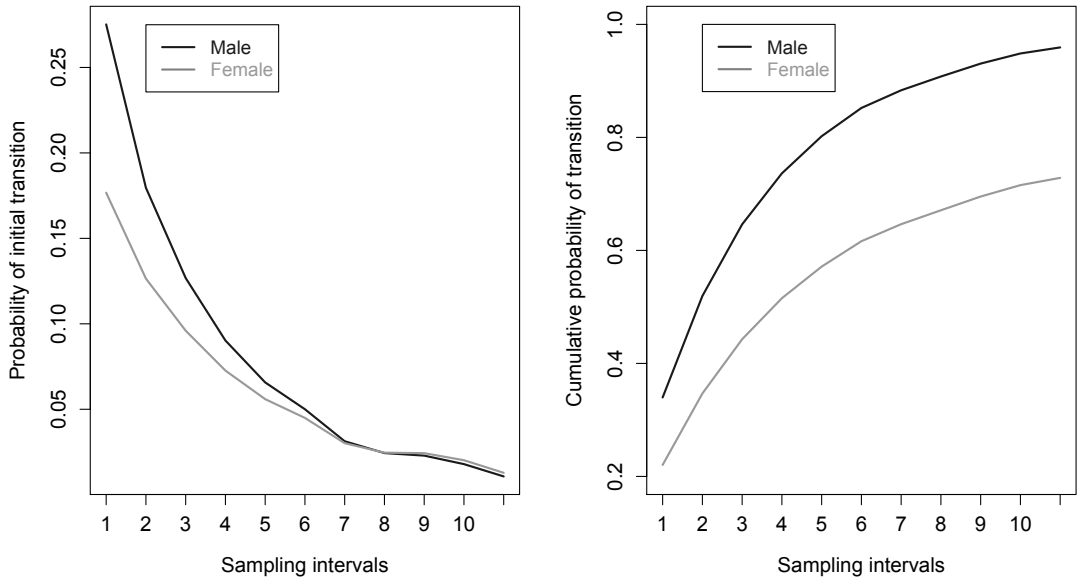

Fig. 4.4. (Left) Results of Markov chain models showing the probability that a house finch first becomes infected with *M. gallisepticum* over the duration of our experiment. (Right) Cumulative probability that an individual becomes infected with *M. gallisepticum* during our experiment. For both figures, black lines represent males, grey lines represent females.

Given that our models identified sex as an important determinant of the probability of infection, we conducted additional analyses in order to describe the magnitude of difference in infection rates of female and male finches over the entire course of spread of disease. Markov chain models show how relatively small probabilities of infection among the sexes between consecutive time periods can result in wider variation over the long term. Per-individual probabilities of initial infection were higher among males at earlier sampling intervals, but through time both male and

female initial probabilities began to asymptote and were not appreciably different (Figure 4.4). The initially higher infection probabilities for males translate to large differences for males and females over the entire course of the experiment, with males ultimately having higher cumulative probabilities of infection than females over the long term (Figure 4.4).

Discussion

These results demonstrate that initial patterns of pathogen distribution within a host group may lead to subsequent differences in rates of spread of infection. Because pathogen distributions are often modulated by the host, the complex interactions of host and pathogen can generate an element of stochasticity regarding patterns of transmission in different populations. To date, our work has concentrated on describing average patterns of pathogen transmission; for example, our recent work showed that *M. gallisepticum* strain differences, rather than host genetics, better predict population-level patterns of infection (Hawley et al. 2010). However, this paper describes our first explicit test of how among-population differences in pathogen loads and the initial conditions of infection can drive variation in infection patterns through time.

We have shown that the distribution of a pathogen among hosts plays an important role in transmission rates. We found that both the amount of pathogen within host groups, and the evenness of its distribution among hosts, are the primary factors that drove infection patterns within our captive host populations. More specifically, a higher pathogen load and a less even distribution, meaning that the

pathogen is concentrated in a small number of hosts, led to higher overall infection rates. This supports earlier literature documenting that superspreaders — either hosts with high pathogen loads or more numerous or frequent contacts — contribute disproportionately to disease dynamics (Lloyd-Smith et al. 2005).

Intrinsic immunological variation among hosts is one potential reason for uneven distribution of a pathogen, and thus, differences in population-level transmission rates. Immunological differences can be attributed to multiple factors, such as genetic variation and hormonal pathways (Ardia et al. 2011). The variation in individual pathogen load likely reflects these host factors, as well as temporal within-host variation as a result of our discrete sampling intervals (Grodio et al. 2008). We did not measure host immunocompetence nor did we determine whether pathogen load is indicative of an 'optimal' immune response among individuals; nevertheless, our results demonstrate that variation in accumulation of pathogen among hosts contributes to infection dynamics over time.

Transmission differences may also result from variation in an individual's propensity to become infected. We found sex-specific differences in transition probabilities, wherein males have higher infection rates than females. Suggesting similar sex-specific patterns, Hawley et al. (2007b) found that males tended to have higher disease severity and a longer duration of infection. Much work has been devoted to understanding the physiological and behavioral reasons for this dichotomy. Two recent reviews of sex-specific hormonal influences and behavior illustrate the complexities inherent in understanding why we may find sex-based immunological differences, or even none at all (Ardia et al. 2011, Hawley and Altizer 2011).

Interestingly, multi-state mark-recapture experiments in wild house finches did not detect a sex effect on survival and recapture rates, though this could have been due to lack of power and difficulty in sexing juvenile birds in the field, or because the encountered birds were part of a progressively selected subset of the population throughout the winter season (Faustino et al. 2004). Work discussed in Chapter 1 also failed to detect an effect of sex; however, this experiment had an uneven sex ratio. Because we were able to sex the birds genetically, had robust sampling of both host and pathogen, a reduction in predation and food stress, and a nearly equal sex ratio, our captive-flock observations may reflect the natural consequences of infection due to these underlying physiological differences. Furthermore, our Markov chain models show sex-specific differences in infection rates, indicating that the population sex ratio might influence infection rates on a larger time scale (Figure 4). At the beginning of an epidemic, males have a higher probability of initial infection than females, but these differences even out and asymptote through time (Figure 4a). However, these sex-specific differences in initial infection rates are magnified when we look at the cumulative probability of infection through time. Even though probabilities begin to saturate as sampling intervals proceed, the cumulative probability that a male has been infected remains much higher than the probability for a female (Figure 4b).

By exploring how infection probabilities vary among individuals, these models allow estimation of host-pathogen dynamics that can occur at the population-level (Zipkin et al. 2010). Our estimates of initial and cumulative infection provide insight into the temporal changes in a disease epidemic that we might expect to observe in groups that vary in sex ratio. Estimated rates of infection for wild house finches

generated by Zipkin et al. (2010) were lower than our results, and this could be due to a number of factors. In the original study upon which their estimates were based, infection rates were based solely on presence/absence of conjunctivitis, and we now know that disease presence is not an accurate predictor of infection (Faustino et al. 2004, Conn and Cooch 2009). Additionally, because *M. gallisepticum* can exhibit density-dependent transmission patterns (Hochachka and Dhondt 2000), wild birds on average may not associate at densities as high as those in our aviaries.

Not important in determining infection rates was the number of infected hosts; in our candidate model set; models including the number of infected hosts had minimal support. Our weekly measurement of infected host numbers corresponded to measures of frequency dependent transmission, wherein pathogen transmission depends on the proportion of infected hosts in the population (we could not measure density-dependence because in our closed systems we did not vary host density). These results are similar to previous analyses of house finch population dynamics before and after the emergence of *M. gallisepticum* in which we found density-dependent regulation of hosts (Hochachka and Dhondt 2000).

We also found no support for differences in the transition rates between aviaries with tube and with platform feeders. Despite previous conflicting results finding high bird mortality at sites with platform feeders (Brittingham and Temple 1986) but higher incidences of mycoplasmal conjunctivitis at sites with tube feeders (Hartup et al. 1998), in our experimental setting we find no evidence for any such differences in infection rates. Perhaps higher prevalence at tube feeders found by Hartup et al. (1998) could have been a result of behavioral differences of house

finches. Hawley et al. (2007) found that infected house finches feed for longer bouts of time than uninfected birds, and these behavioral differences may be affected by feeder structure. Feeders and shared resources in other systems have been found to alter the contact structure of individual interactions (Leu et al. 2010) or influence aggregations of parasites, and thus parasite avoidance behaviors (Roper et al. 2002, Reckardt and Kerth 2007). However, any behavioral differences or changes in social structure resulting from resource provisioning were not strong enough to influence the overall effect of the feeder type on rates of *M. gallisepticum* infection.

To better understand broad scale patterns of disease dynamics, we must account for pathogen-specific characteristics, while still recognizing that these patterns will likely vary among host groups. Ideally, future models would account for all heterogeneities of both host and pathogen to accurately predict downstream patterns of infection; but this remains a complex task. Further work is needed to better understand what constitutes an infectious load, or dose, within an individual, particularly in the wild. This experiment emphasizes the importance of pathogen-mediated effects on host infection patterns, but also illustrates the need for a better understanding of proximate host responses to pathogen variation and the ultimate evolutionary consequences of such pathogen variation within the host population.

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CHAPTER 5

UNDERSTANDING THE ORIGIN OF SEASONAL EPIDEMICS OF MYCOPLASMAL CONJUNCTIVITIS*

Abstract

1. Many host-pathogen systems show regular seasonal oscillations.

2. Seasonal variation of mycoplasmal conjunctivitis prevalence in house finches is an example of such oscillations.

3. An annual pulse of *Mycoplasma gallisepticum*-naïve juveniles increasing the number of susceptibles, seasonal changes in flocking behavior increasing transmission rate, and a gradual loss of resistance to reinfection with time are sufficient to model the observed seasonal variation in disease prevalence. Nevertheless, experiments are needed to test the underlying mechanisms.

4. We carried out an 18 month experiment with small groups of birds in large aviaries to test two hypotheses.

5. To test the first hypothesis, that an influx of naïve juveniles in a group of recovered adults is sufficient to cause an outbreak, we added 8 juveniles to a group of 11 adults that had recovered from an earlier infection. In all three replicates juveniles became infected, but only after some of the adults relapsed.

6. To test the second hypothesis that reintroduction of *M. gallisepticum* into a multi-age group of previously exposed, but fully recovered house finches causes a new outbreak, we inoculated two birds in each group in March of the 2nd year. Contrary to

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what happens in the wild at that time disease prevalence increased rapidly after reintroduction of *M. gallisepticum*.

7. We conclude that recovered adults with no physical signs can initiate an epidemic and transmit *M. gallisepticum* to naïve house finches, and that the reintroduction of *M. gallisepticum* is sufficient to cause a new outbreak, even at a time of the year when mycoplasmal conjunctivitis is low in free-living birds. Date, as such, seems to be less important to explain seasonal variation in conjunctivitis than the presence of naïve juveniles or the introduction on *M. gallisepticum*.

8. Seasonality in outbreaks is most likely tightly linked to seasonal variation in bird movements and behavior.

Introduction

Many host-pathogen systems show regular, often seasonal, oscillations. The precise mechanisms that drive these are often not completely understood and require, if possible, experimental studies (Altizer et al. 2006). Seasonal variation can be driven both by external factors such as changes in climate or food and by internal factors such as variations in immune competence (Hawley and Altizer 2011). Mycoplasmal conjunctivitis in house finches is such a system with strong seasonal variation (see Appendix Figure A.1). Conjunctivitis prevalence is minimal — often zero in local populations — during the breeding season (April-July) in all regions where studied (Dhondt, Tessaglia and Slothower 1998; Hartup et al. 2001a; Altizer et al. 2004a; Altizer, Hochachka and Dhondt 2004b; Faustino et al. 2004; Nolan, Roberts and Hill 2004; Dhondt et al. 2005; Dhondt et al. 2006; Jennelle et al. 2007). In late summer and

fall, prevalence increases gradually reaching a maximum in October to November. The exact timing and amplitude of the fall maximum varies geographically, likely due to latitudinal variation in the timing of the breeding season and the period when juveniles join the population (Altizer et al. 2004b). In December, prevalence reaches a new low, followed by a second smaller peak in late February and early March, after which prevalence returns to the breeding season minimum. Seasonal variations in the prevalence of house finch conjunctivitis have been attributed to a combination of factors. The two most commonly proposed explanations for the increase in disease prevalence are (1) the seasonal influx of the cohort of susceptible juveniles in late summer; (2) seasonal changes in social behavior, whereby birds aggregate in late summer and fall (mostly juveniles), and winter (all birds) which increases the probability of transmission. The December minimum has been attributed to recovered birds being temporarily resistant to re-infection, and the late winter peak is probably caused by a gradual loss of immunity among birds that had recovered from an earlier infection (Hosseini, Dhondt and Dobson, 2004). Although there is no direct experimental evidence for any of these explanations, the models of Hosseini et al. (2004) strongly suggest that it is the combination of seasonal changes in social aggregation and the gradual loss of immunity — and hence resistance to reinfection — that drive seasonal variation in disease prevalence.

A separate unresolved problem central to the dynamics of house finch conjunctivitis concerns the source of *M. gallisepticum* that starts the late summer epidemics in locations where the disease had disappeared. Various non-exclusive possibilities are: (1) previously exposed, but recovered adults, have no physical signs

but remain infectious (Nolan et al. 2004), or relapse and become infectious again; (2) *M. gallisepticum* is reintroduced into local populations by dispersing or migrating individuals from other locations in which pockets of *M. gallisepticum* survived; (3) given that *M. gallisepticum* has been documented in many bird species coexisting with house finches (Hartup et al. 2001b; Mikaelian et al. 2001; Farmer, Hill and Roberts, 2005; States, Hochachka and Dhondt, 2009) other bird species could function as a reservoir, and reintroduce *M. gallisepticum* into house finch populations.

The objective of the experiment described in this paper is to test hypotheses (1) and (2) concerning the persistence and/or reintroduction of *M. gallisepticum* into host populations. To do that we introduced *M. gallisepticum* in small captive flocks of house finches in three replicated large aviaries (two octagonal cages connected by a corridor; see Appendix Figure A.2). The birds were kept at a low density in cage systems that permitted individuals to avoid one another. We followed the epidemic in each group, and allowed the birds to breed. When all adult birds had finished breeding in September and showed npo physical signs we added a group of naïve juveniles to each aviary but did not actively reintroduce *M. gallisepticum*. This would mimic a situation in which a seasonal pulse of naïve juveniles joins a local previously exposed population that has no physical signs (hypothesis 1). The null hypothesis was that adding naïve juveniles to recovered birds would not cause a new epidemic and that the juveniles would not develop disease; the alternate hypothesis was that naïve birds would become infected. For that reason we did not keep one of the three groups as a control. Even if in only one of the three groups a single naïve juvenile became infected hypothesis 1 would be supported, as it would show that previously exposed but

recovered adults could transmit *M. gallisepticum*.

In March, at a time when disease prevalence in the wild is low and declining, we re-introduced *M. gallisepticum* in the fully recovered mixed-age groups to test hypothesis 2. This latter experiment made it possible to separate seasonally driven changes in disease prevalence from changes caused by the reintroduction of *M. gallisepticum* at a time when disease prevalence is naturally in decline. Because for logistical reasons we only had three replicates we decided not to keep one as a control, but to reintroduce *M. gallisepticum* in all three groups. In fact, we thought it unlikely that *M. gallisepticum* would spread in a previously exposed flock at that time of year, and wanted to increase the likelihood of a new epidemic by having three replicates. The 'control' would be the multiple observations that at that time of year *M. gallisepticum* prevalence is low and decreasing (Dhondt et al. 1998; Hartup et al. 2001a; Altizer et al. 2004a; Altizer et al. 2004b; Nolan et al. 2004; Dhondt et al. 2005; Dhondt et al. 2006; Jennelle et al. 2007).

Materials and Methods

Source of the birds

All birds were juvenile house finches trapped in Tompkins County, NY under USGS Bird Banding Lab permit #23513, and housed at Cornell under permit #2006-094 from the Cornell Institutional Animal Care and Use Committee. The birds for the initial experiment were trapped in summer 2007. The birds that were added in September 2008 were trapped in the summer of 2008.

House finches were held in quarantine for a minimum of two weeks in

individual cages and fed *ad libitum* a pelleted diet (Roudybush Maintenance, Cameron Park, CA, USA). Birds were tested for exposure to *M. gallisepticum* by examination for eye lesions, presence of the bacterium by quantitative polymerase chain reaction (qPCR), and antibodies by Rapid Plate Agglutination assay (RPA) during the first season or enzyme-linked immunosorbent assay (ELISA) during the second and third seasons. Only birds that were negative for all tests were used in the experiments.

Experimental aviaries

Inside a large barn with artificial light, but no heating, we built three identical aviaries, each consisting of two octagonal rooms (hereafter octagon) connected by a corridor of 1.5 m long x 2.40 m high and 1.20 m wide. Each octagon had eight panels of 1.20 x 2.40 m and a maximum height of 3 m in the center. The ground surface area was 6.87 m^2 and the volume 17.87 m^3. Thus each group of birds could move freely within a total volume 40.1 m^3 (see Appendix, Fig. 2). Given that the aviaries were inside a closed barn wild birds could not come into contact with the experimental birds, and could, therefore, not be responsible for reintroducing *M. gallisepticum* into the system.

Each aviary was equipped in the same way with two large six-port tube feeders (re-filled daily with Roudybush Maintenance) hung from the center; two artificial Christmas trees placed in a corner; and several plastic perches attached to the walls. Close to one of the perches we also provided an ceramic heating lamp during winter. On the aviary walls, at about 1.80 m height, we hung five artificial Christmas wreaths that birds used for roosting and nest building. A water bath, heated by a heating lamp

in winter, was cleaned and refilled every day. The cement floor was cleaned twice weekly. During the breeding season nesting material was provided in each aviary.

Experiments

After keeping 11 *M. gallisepticum* -naïve juvenile house finches at low density with *ad libitum* food in each aviary system from October 2007 to February 2008, we introduced *M. gallisepticum* in each group by inoculating one index bird chosen at random on 25 February 2008. After instilling 0.05 ml of inoculum in each eye the birds were held in a paper bag for 10 minutes to ensure full absorption of the inoculum; inoculated birds were then re-released into their respective group. We used a 7^{th} in vitro broth passage from the original *M. gallisepticum* house finch isolate 7994-1 (Ley, Berkhoff and McLaren 1996).

We followed horizontal transmission through the group from March through June 2008 (period 1), when all birds had recovered, and allowed the birds to breed by providing them nest material and nest sites. Captive-born juveniles were removed from the aviaries. After the breeding season was over and all birds no longer displayed physical signs, we added 8 unrelated *M. gallisepticum*-naïve juveniles (born in 2008) to each group of adults, creating a mixed age flock consisting of previously exposed, but recovered adults, and previously unexposed juveniles, a situation as would normally exist among free-living birds (period 2). Two juveniles in aviary A died shortly after introduction of unknown causes and were not replaced. To test the first hypothesis we monitored a possible re-emergence of *M. gallisepticum* until mid-February 2009. To test the second hypothesis we then reintroduced *M. gallisepticum*

into each flock by inoculating one adult and one juvenile in mid-March 2009 and studying horizontal transmission until June 2009 (period 3), when the experiment was terminated. Dominance positions of all birds were determined from interactions at feeders and expressed as proportion interactions won.

Measures of infection with M. gallisepticum

Infection by *M. gallisepticum* was measured weekly by one of three criteria: birds developed conjunctivitis whereby eye lesion severity received a score of 0 (no lesions)-3 (severe lesions) (Sydenstricker et al. 2005); conjunctival swabs (and choanal swabs biweekly) were individually tested for *M. gallisepticum* using qPCR (Grodio et al. 2008); the presence of *M. gallisepticum* -specific antibodies in a blood sample was tested every other week. To test whether birds were seropositive, we used RPA to test for presence/absence of antibodies during the period 25 Feb to 2 June 2008; for subsequent periods we quantified the presence of IgY antibodies using ELISA (Grodio et al. 2009). In our earlier studies on horizontal transmission we observed that birds were not always positive for *M. gallisepticum* by all three criteria (Sydenstricker et al. 2006). We therefore considered a bird infected if any one of the three criteria was satisfied. In order to estimate the date of first exposure to *M. gallisepticum* we only used qPCR results and eye scores, and used the first date a bird was positive as the date of transmission.

We calculated 'survivorship' of healthy birds from the beginning of each period using a Kaplan-Meier survivorship analysis, as data were censored. Analyses were performed using Statistix 8, Analytical Software, Tallahassee FL, USA. We

compared survivorship between groups using all methods provided by Statistix 8 that allow comparisons of multiple groups, as different tests make somewhat different assumptions regarding the data. Given that in all analyses (the Gehan-Wilcoxon test, the Peto-Wilcoxon test, and the Log-Rank test) the results were always very similar, we will report the results of the Log-Rank test only, as it allows data to be censored and does not assume a particular survival function.

Results

Horizontal transmission in same-age groups of house finches naïve for M. gallisepticum*: February-June 2008 (Period 1)*

Each of the three birds inoculated on 25 February 2008 (one per group) developed eye lesions one week post-inoculation (PI). Lesions remained visible until week 12, 7, and 8 PI and the presence of *M. gallisepticum* was confirmed in conjunctival swabs until weeks 8, 6 and 8 PI in aviaries A, B and C, respectively (Table 5.1). Therefore we assumed that transmission from the index bird could have taken place between the development of lesions and the termination of bacterial detection from eye swabs.

In all three aviaries the epidemic was very rapid and 28/30 individuals became infected by *M. gallisepticum*. Two weeks after inoculation of the index bird, *M. gallisepticum* was detected in 16 (53%) of the naïve birds. The proportion of birds in the three aviaries that were either had physical signs or tested positive for *M. gallisepticum* on a given date remained high for another week, after which it decreased rapidly (Figure 5.1). After some fluctuations, *M. gallisepticum* was detected in only one naive bird in week 12 PI and in no birds by week 14, when sampling was

terminated until September. Thus, in all three aviaries nearly all birds became exposed to *M. gallisepticum*, but no birds died following infection.

Table 5.1. Cases of econdary transmission in period 1. Number of naïve individuals in each aviary that developed conjunctivitis or *M. gallisepticum*-specific antibodies, or in which *M. gallisepticum* was detected through qPCR from conjunctival swabs. There were 10 naïve birds present in each group.

Aviary	No. of individuals with conjunctivitis	No. of individuals with *Mycoplasma gallisepticum* detected by qPCR	No. of individuals with MG-specific antibodies	No. of individuals exposed by any method
A	3	9	4	9
B	4	7	6	10
C	4	4	6	9
Total	11	20	16	28

Horizontal transmission in mixed -age groups of house finches: September 2008 - March 2009 (Period 2).

On 17 September 2008, after all individuals exposed to *M. gallisepticum* in Period 1 did not have physical signs and tested negative for bacterial shedding we added 8 naïve juveniles to each aviary. At this time 28/30 birds had antibody titers below the level of infected birds (0.07). In Aviary A two individuals, including the index bird, still carried elevated levels of antibodies (above 0.10).

Although we did not actively reintroduce *M. gallisepticum* into the aviaries a total of 6, 6 and 4 adults in aviaries A, B, and C, respectively, developed evidence for *M. gallisepticum* infection (Figure 5.2). Of these birds 2, 1, and 3 carried *M.*

gallisepticum detectable by qPCR in the conjunctiva, and thus could have been infectious. In the other individuals we either observed eye lesions or detected

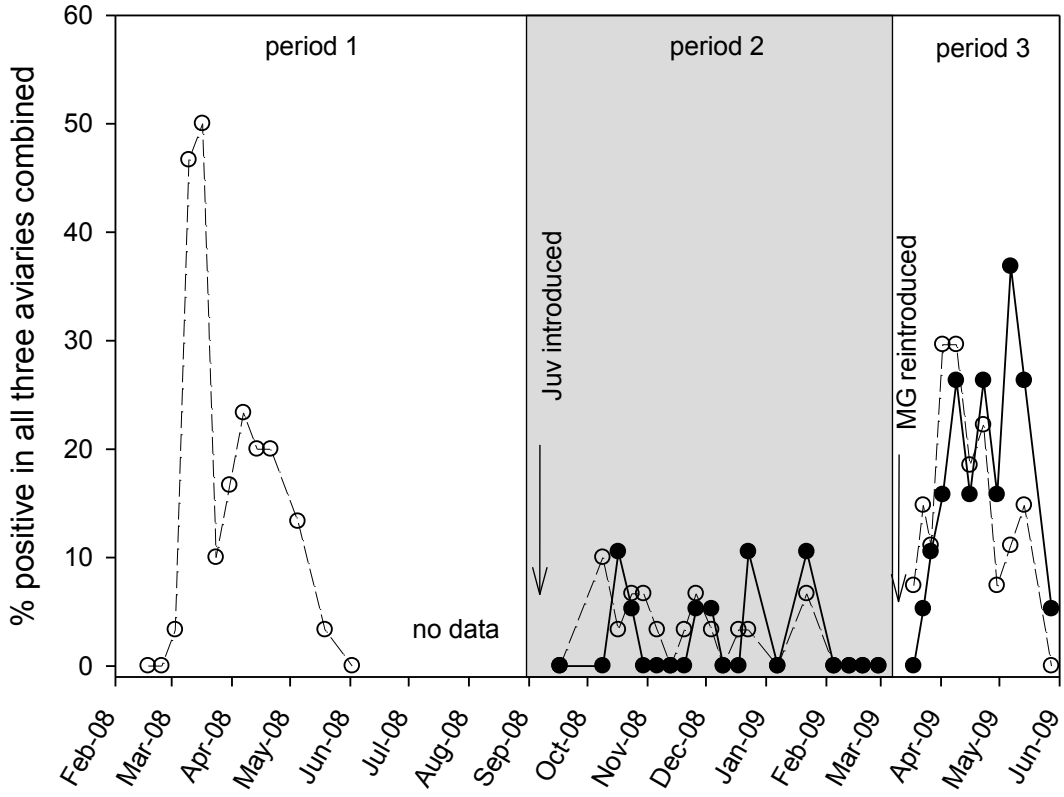

Figure 5.1. Percentage of individuals positive for *M. gallisepticum* on each sampling date between February 2008 and June 2009 (adults n = 30; juveniles n = 22; in three aviaries). Open symbols: birds born in 2007, and hence adult in 2009; filled symbols: birds born in 2008, and hence juvenile until June 2009. At the onset of period 1 *M. gallisepticum* was introduced by inoculating one individual in each of the three aviaries; at the onset of period 2 eight naïve juveniles were added to each group of recovered adults; at the onset of period 3 *M. gallisepticum* was re-introduced into each aviary by inoculating one adult and one juvenile in each aviary.

M. gallisepticum-specific antibodies. The dominance rank of adults that relapsed did not differ from those that did not relapse in any of the three groups (t-test: aviary A: $t_8 = 0.58$, $P = 0.58$; aviary B: $t_8 = 0.51$, $P = 0.62$; aviary C: $t_8 = 0.30$, $P = 0.77$). In each of the three aviaries some of the naïve juveniles became infected during this period (3/8; 5/8; 2/6, respectively), as measured by one of the three criteria (Figure 5.2). Six of the juveniles were qPCR positive, and would have the potential to further transmit *M. gallisepticum*.

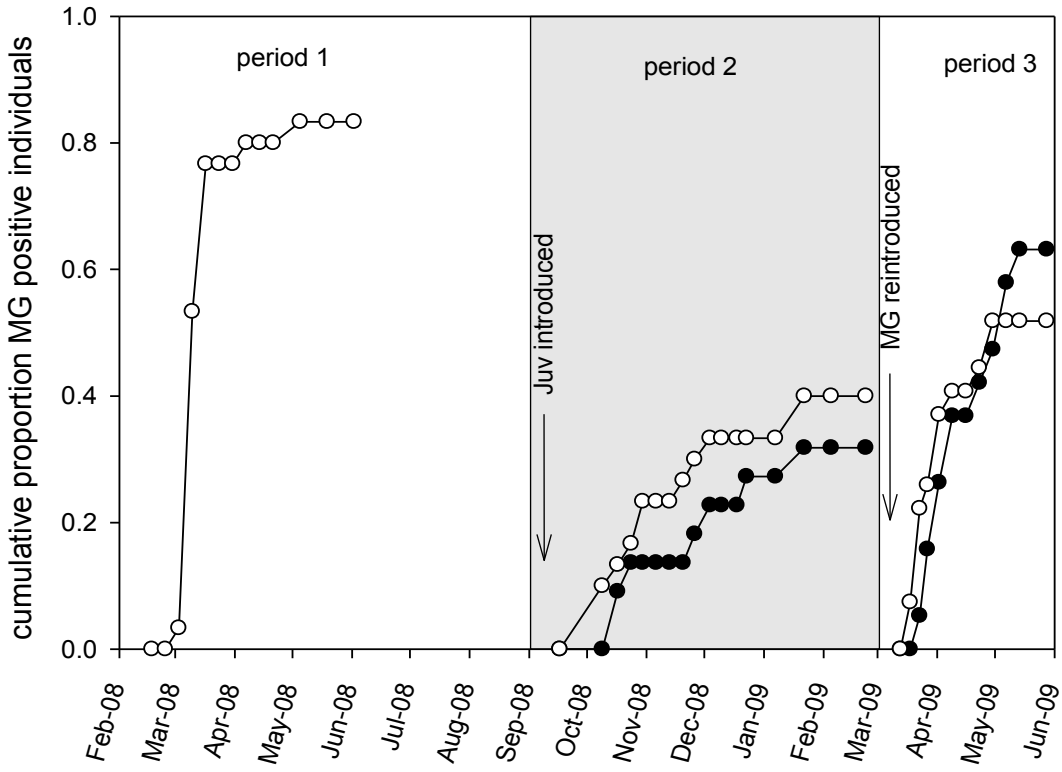

Figure 5.2. Cumulative proportion of house finches infected by *M. gallisepticum* in each of three periods.

We explored the possible origin of the infection by analyzing the dates at which the first bird in an aviary tested qPCR positive, and in all cases this was an adult. In aviary A, the first adult, was qPCR positive on 9 October, and the first infected juvenile (qPCR positive) was observed one week later. Another adult developed conjunctivitis on 17 October, and another juvenile had mild conjunctivitis in one eye on 24 October. In one more adult we detected *M. gallisepticum* in a conjunctival swab on 22 January 2009. In aviary B, one adult was positive for *M. gallisepticum* on 17 October and three adults had eye lesions on 24 October, and on 20 and 26 November. Juveniles were qPCR positive for *M. gallisepticum* on 26 November, 23 December, and 22 January. In aviary C, one adult was qPCR positive on 9 October. The first infected juvenile (qPCR positive) was detected one week later, on 17 October. The other adults were qPCR positive on 30 October 2008, 18 December 2008 and on 22 January 2009 respectively.

Not all adults that had recovered by September 2008 showed signs of relapse during the 2nd period. During this period the duration of infections in an individual was short; although 12/30 (40%) adults and 7/22 (32%) juveniles showed evidence of an active infection (qPCR or eyescore) on any one date not more than 10% of adults and 10.6% of juveniles showed signs of infection (Figure 5.1).

Adding juveniles to a group of previously exposed adults with no physical signs in an aviary thus resulted in a low-level outbreak of *M. gallisepticum* in each of the three systems.

Effect of new introduction of M. gallisepticum *in a previously exposed, mixed-age*

flock: March-May 2009 (Period 3)

On 13 March 2009 one adult and one juvenile in each aviary were inoculated with the same strain of *M. gallisepticum* that had been used in the first period of the experiment. In Table 5.2 we summarize the history of the birds that were used as index birds in the third period. In brief, in all three adults *M. gallisepticum* DNA had been detected by qPCR in the first period; in period 2 all birds with the exception of the

Table 5.2. Health history of the 6 index birds for the period 3 experiment; they were inoculated on 3 March 2009.

Aviary	Age	Period 1	Period 2	Period 3
A	Adult	PCR	Eyescore on 30 Oct (1 in one eye)	Died 5 days after reinoculation
A	Juvenile	nd	Negative	Eye; PCR; AB
B	Adult	PCR	Negative	Eye; PCR; AB
B	Juvenile	nd	Negative	Eye; PCR; AB
C	Adult	PCR	Negative	Eye; PCR; AB
C	Juvenile	nd	Negative	Eye; PCR; AB

Negative: no evidence for *M. gallisepticum* exposure using any of the three criteria; Eye: eyescore >0 on at least one date; PCR: *M. gallisepticum* detected using qPCR on at least one date; AB: antibodies detected using ELISA (or RPA in period 1) at least once. Thus the adult in aviary B was PCR+ during Period 1, remained negative for all three criteria in Period 2, and developed conjunctivitis (eye), became PCR+ and developed MG-specific antibodies during Period 3. The juveniles were not yet born in Period 1; hence, there are no data (nd) for them.

adult in Aviary A had shown no signs of exposure to *M. gallisepticum*; and in period 3 all index birds responded strongly to reinoculation: they developed eye lesions, tested positive for *M. gallisepticum* for several weeks, and developed *M. gallisepticum*-specific antibodies. The adult index in aviary A died, for unknown reasons, 5 days

after inoculation, so only the juvenile index could be responsible for the new epidemic in that aviary. As shown in Figure 5.1 the reintroduction of *M. gallisepticum* in a flock caused a strong increase in the spread of disease in both adults and juveniles.

The rate of horizontal transmission, as measured by survivorship, differed significantly between periods (Figure 5.3): the probability to become infected at a given time was highest during period 1, intermediate during period 3, and much lower during period 2 (Log-Rank test, combined data: $c^2 = 28.44$, d.f. = 2; $P < 0.0001$; period 1 versus period 3: $c^2 = 6.05$, d.f. = 1; $P = 0.014$; period 3 versus period 2: $c^2 = 10.39$, d.f. = 1; $P = 0.001$). We combined the observations of the two age groups in an aviary and of the three aviaries in each period for these calculations because, groups were not significantly different using the Log-Rank test when applying a sequential Bonferroni correction (*aviary effect* :period 1: $c^2 = 3.83$, d.f. = 2; $P = 0.15$; period 2, all ages: $c^2 = 0.26$, d.f. = 2; $P = 0.79$; period 3, juveniles : $c^2 = 3.77$, d.f. = 2; $P = 0.15$; period 3, adults: $c^2 = 7.40$, d.f. = 2; $P = 0.02$. We have 4 tests in which we evaluate aviary effects. A sequential Bonferroni correction would require a lowest P-value $\leq 0.05/4=0.0125$. The lowest P-value is 0.02 (period 3; adults). We thus conclude that there are no aviary effect. *age effect:* period 2, all aviaries: $c^2 = 0.47$, d.f. = 2; $P = 0.49$; period 3, aviary A: $c^2 = 2.01$, d.f. = 1; $P = 0.15$; period 3,aviary B: $c^2 = 0.54$, d.f. = 1; $P = 0.46$; period 3,aviary C: $c^2 = 4.86$, d.f. = 1; $P = 0.03$; A sequential Bonferroni correction would require that the lowest P-value $\leq 0.05/3 = 0.0167$. The lowest value is 0.03 (aviary C). We thus conclude that there is no age effect.)

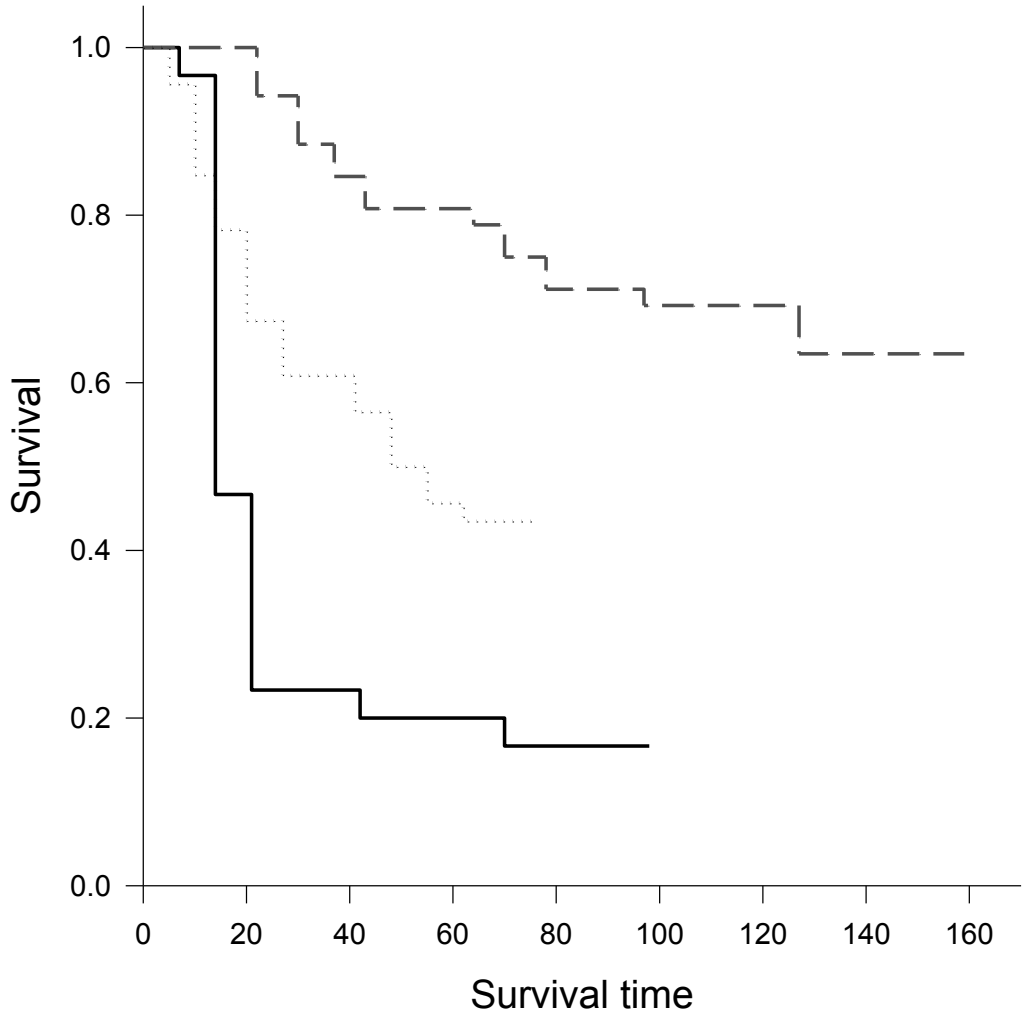

Figure 5.3. 'Survivorship' (probability to remain uninfected) from a Kaplan-Meier survivorship analysis of three groups of house finches infected by *M. gallisepticum* in each of three periods. Note that the probability to be uninfected on a given date was highest during the 2nd period (long dash, intermediate during the 3rd period (dotted line), and lowest during the 1st period (solid line).

Discussion

Origin of the fall epidemic – Hypothesis 1

This experiment, with a low density of house finches in a large aviary, confirmed earlier ones that when *M. gallisepticum* is introduced into a captive flock a rapid epidemic follows, and most individuals become infected. The lower density of birds in aviaries in which the birds could avoid one another was a deliberate attempt to try to mimic conditions of free-living birds, and may have been responsible for the relatively rapid recovery of all birds. At lower density birds will interact less frequently, be less stressed and therefore more immunocompetent (Hawley 2006), and are therefore less likely to become reinfected or relapse after recovery. The main difference between this experiment and a similar one carried out in 2002 (Sydenstricker et al. 2006) was the space available to the birds. In the earlier experiment we kept 11 house finches in a rectangular aviary of 11.7 m^3 (1.8m x 3.6 m x 1.8 m), or about 1 bird per m^3. In an aviary of that size it was difficult for birds to avoid one another. In this experiment we kept 11 birds in a volume of 40.1 m^3, or about 3.7 m^3 per bird, a space almost 4 times larger. In this setup birds could avoid other individuals easily as birds had no problems in flying through the corridor from one octagon to the other. In the earlier experiment, disease prevalence (measured by eyescore) increased rapidly, similar to results for the current experiment, but remained high at around 40% of the birds until week 21 PI, when the experiment was terminated. In the current experiment the proportion of infected birds (as measured by eyescore or by qPCR) already started to decrease on week 4 PI, and all birds had recovered by week 14 PI. In both experiments we used the same source of *M.*

gallisepticum, although in 2002 we used a 6th in vitro broth passage from the original *M. gallisepticum* house finch isolate, 7994-1, while in 2008 we used a 7th passage. We do not believe that the difference between the results of these two experiments is primarily due to this difference in number of passages of the pathogen, although we have not tested this rigorously.

In the second phase of our experiment a relapse of adults followed by transmission to naïve juveniles occurred in all three aviaries (Figure 5.1). In each aviary, adult birds showed signs of infection by *M. gallisepticum* before juveniles became infected. This indicated that in each aviary at least one adult relapsed, became infectious and was the probably source of the new epidemic that also caused infection in some naïve juveniles. Transmission (or relapse) was slow and occurred with low frequency among both the previously exposed adults and the naïve juveniles that were added to the groups. Although on any date only about 10% of birds (both adults and juveniles) were infected as measured by eyescore or by qPCR (Figure 5.1), a total of 7 of 22 juveniles (32%) and 12 of 30 (40%) adults became infected during the 21 weeks following the introduction of the naïve juveniles.

We can thus conclude that adults with no physical signs were able to transmit *M. gallisepticum* to naïve juveniles that flock with them, which was the primary objective of this experiment. The fact that this was observed in all three replicates suggests that this would be a normal event in the wild. Our experiment did not allow us to determine what caused the September epidemic in the aviaries. It could be that, in any situation, some previously exposed but recovered adults relapse after the breeding season and during molt. In chickens, for example, induced molt causes

immunosuppression (Golden et al. 2008). In our system, however, this is unlikely to be the primary factor as in earlier experiments recovered individuals held for over a year in individual cages did not relapse, but did develop physical signs again after being reinoculated with *M. gallisepticum* (Sydenstricker et al. 2005). It could be that the increase in density in the aviary by adding juveniles triggered the relapse, because of an increase in stress causing reduction in immunocompetence, but this was not measured. It is not likely that competition for food played a major role, since food was provide *ad libitum*. Nevertheless the number of birds per feeder does affect stress and immunocompetence (Hawley, Lindstrom and Wikelski, 2006) and adding birds, even with unlimited food, might have stressed the birds. Another possible source of the *M. gallisepticum* that re-emerged in Period 2 could have been the naïve juveniles that we introduced. As a reviewer pointed out, one could argue that not all the juveniles added in period 2 (although tested and found to be *M. gallisepticum* negative) were truly previously unexposed as they had not been reared in captivity under controlled conditions. Perhaps some juveniles had been exposed between birth and field capture, since most adult birds at the beginning of period 2 tested *M. gallisepticum*- negative for all test procedures though they were known to have been exposed in period 1. We believe this to be unlikely for two reasons: first, in all groups adults relapsed at least one week before any evidence of *M. gallisepticum* infection in juveniles; second, when captured in July-August the juveniles were about 1-2 months old. Had they been exposed to *M. gallisepticum* in the wild they would either develop disease when kept in isolated cages during the quarantine period, or would still have *M. gallisepticum*-specific antibodies in their blood when tested after capture and before being

introduced into the aviary. Finally, and although we did not have a control group, it is not possible that *M. gallisepticum* would have been introduced from wild birds, as the aviaries were inside a closed barn, and hence completely isolated from free-living birds.

Origin of the spring epidemic

In free-living populations of house finches birds prepare to start breeding in March, after which disease prevalence rapidly declines (Altizer et al. 2004b). In contrast to the seasonal decline in disease prevalence in the wild the re-introduction of *M. gallisepticum* into the captive multi-age group previously exposed to the same isolate resulted in a rapid increase in disease prevalence in each of the three flocks. The rate of increase in prevalence was slower than in the first period when *M. gallisepticum* was introduced into a naïve group of birds, but was clearly more rapid than during the second period when the epidemic originated from relapse of adults. During period 2 the number of successive samples that were qPCR positive in a single individual was never more than 1 for adults and than 2 for juveniles, implying that infections were very mild and therefore short-lived. After *M. gallisepticum* was reintroduced in March some individuals were qPCR positive for up to 9 weeks (adults) and up to 7 weeks (juveniles). Reintroduction of *M. gallisepticum* in a previously exposed group is thus sufficient to cause a disease outbreak, in support of hypothesis 2.

Seasonality as a driver?

The observation that the re-introduction of *M. gallisepticum* into a previously exposed multi-age group (as would exist in nature) caused a new outbreak indicates that seasonal increases in disease prevalence could result from either the re-introduction of *M. gallisepticum* in local populations by infectious individuals or through relapse of birds that previously recovered. House finch movements start in mid to late summer (post-fledging dispersal), and are followed by a partial migration in October. This coincides with the time when disease prevalence increases in the late summer and fall. These movements, therefore, likely play an important role in the increase of prevalence at that time, because *M. gallisepticum* is moved and introduced into groups of largely naïve birds. Further, it cannot be excluded that birds returning from wintering grounds in February-March are in part responsible for an increase in disease prevalence at that time (Able and Belthoff 1998). It is also possible that the stress of migration and establishing breeding territory would cause a relapse of recovered birds (Altizer, Bartel and Han 2011).

The fact that we observed a new epidemic in March, after re-introducing *M. gallisepticum* in the groups, makes it possible to conclude that it is not seasonality per se (changes in food abundance, photoperiod, temperature, etc.) that causes seasonal variation in disease prevalence in house finches, but rather changes in social behavior and movements associated with certain seasons that bring naïve birds into contact with infectious individuals, and that this can result in an outbreak.

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CHAPTER 6

SPATIAL VARIATION IN AN AVIAN HOST COMMUNITY: IMPLICATIONS FOR DISEASE DYNAMICS[†]

Abstract

Because many pathogens can infect multiple host species within a community, disease dynamics in a focal host species can be affected by the composition of the host community. We examine the extent to which spatial variation in species' abundances in an avian host community may contribute to geographically-varying prevalence of a recently emerged wildlife pathogen. *Mycoplasma gallisepticum* is a pathogen novel to songbirds that has caused substantial mortality in house finches (*Carpodacus mexicanus*) in eastern North America. Though the house finch is the primary host species for *M. gallisepticum*, the American goldfinch (*Spinus tristis*) and northern cardinal (*Cardinalis cardinalis*) are alternate hosts, and laboratory experiments have demonstrated *M. gallisepticum* transmission between house finches and goldfinches. Still unknown is the real world impact on disease dynamics of variation in abundances of the three hosts. We analyzed data from winter-long bird and disease surveys in the northeastern United States. We found that higher disease prevalence in house finches was associated with higher numbers of northern cardinals and American goldfinches, although only the effect of cardinal abundance was statistically significant. Nevertheless, our results indicate that spatial variation in bird communities has the potential to cause geographic variation in disease prevalence in house finches.

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The prevalence of any disease will vary through time and across space. Host densities or interaction rates will vary with the suitability of the environment, leading to different density- or frequency-dependent rates of transmission of pathogens (Rudolf and Antonovics, 2005). The pathogens themselves may also be directly affected by variation in environmental conditions if the pathogens exist outside their hosts for any length of time. Additionally, many pathogens can live in multiple host species and each of these hosts may vary in competency and in its role as a reservoir (Keesing et al. 2006; Craft et al. 2008). With this last case, the composition of host communities can affect the prevalence of disease in any single host species (Ostfeld and Keesing, 2000; LoGiudice et al. 2003; Dobson, 2004; Peixoto and Abramson, 2006). As a result, spatial variation in either the composition of host assemblages or relative abundances of hosts can cause spatial variation in disease prevalence. In this paper, we quantify the relationship between abundances of three bird species and prevalence of disease in one of the hosts in nature, using data collected across the northeastern United States.

Our focal host is the house finch (*Carpodacus mexicanus*), a small songbird native to western North America but now widespread across the eastern United States. House finches are susceptible to the bacterium *Mycoplasma gallisepticum*, which causes conjunctivitis in the finches. *Mycoplasma gallisepticum* emerged as a pathogen of house finches in February 1994 (Fischer et al. 1997), spreading through house finch populations in eastern North America within a few years (Dhondt et al. 1998) and reducing house finch populations up to 60% (Hochachka and Dhondt,

2000). Recent work implicates bird feeders (fomites) as one of the possible modes of *M. gallisepticum* transmission within and among species (AA Dhondt et al. 2007).

House finches are the primary host species for the "house finch" strain of *M. gallisepticum*, but we know that American goldfinches (*Spinus tristis*) and northern cardinals (*Cardinalis cardinalis)* are secondary hosts (Hartup et al. 2001). Further, experimentally-infected American goldfinches developed conjunctivitis and were able to transmit the bacterium to house finches, suggesting that goldfinches are reservoirs in the wild (Dhondt *et al.* 2008). Northern cardinals have tested positive for DNA of and antibodies against *M. gallisepticum*. While we expect that the abundance of these alternate host species at a site will be related to disease prevalence in house finches, a given increase in alternate host abundance may have a different effect on disease prevalence in house finches than the same change in house finch abundance.

Our data came from two citizen science programs based at the Cornell Laboratory of Ornithology: Project FeederWatch and the House Finch Disease Survey. Project FeederWatch (Wells et al. 1998; Lepage and Francis, 2002) provides bird species abundance data, collected by volunteers who count the number of birds seen at their feeders on multiple two-day periods throughout the winter (e.g., Hochachka and Dhondt, 2006). Counts of zero birds can be inferred for any of our species, because FeederWatch participants report numbers of birds seen for all species on their checklists, which include American goldfinch, northern cardinal, and house finch. The House Finch Disease Survey is largely an adjunct of Project FeederWatch, with participants counting numbers of house finches with conjunctivitis as they count numbers of birds at their feeders (Dhondt et al. 2005). Counts of zero house finches

with conjunctivitis were explicitly reported, not inferred by lack of reporting.

The subset of data that we examined came from the winter seasons (hereafter "seasons") of 2000-2001 to 2006-2007, after *M. gallisepticum* became endemic in house finches throughout our study region (Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, and Vermont). We restricted our data to sites that reported our primary host, the house finch, and further removed data from sites that reported counts of birds within the top five percent for any of the species, because prior experience suggests that a large proportion of these counts are erroneous. This left data from 2,995 sites available for analysis.

We could not use the reported counts of birds as indices of true abundance of birds at a site, because the actual number reported will vary with observer effort (e.g., Hochachka and Dhondt, 2006). Thus, we needed to statistically correct for variation in effort among observations, calculating a predicted count of each species for standardized values of observer effort. We also accounted for within-season variation in counts of birds at feeders (e.g., Hochachka and Dhondt, 2006), and because there is a roughly 14-day incubation period until peak disease severity (KV Dhondt *et al*. 2007) we calculated our standardized counts for dates 14 days before the dates of observation of conjunctivitis. Our counts of birds were over-dispersed (excessive numbers of zero-counts to fit normal or log-normal models) so we used a two-step regression to fit models and calculate predicted values. The first step was a logistic regression, in which presence or absence of each species at a site on a given date was modeled as a function of season, observation date, site, observer effort, and bird

species. When absence was predicted in the first step (probability <0.5 of presence), we used a predicted value of zero for that site and date. When presence was predicted in the first step, we then fit this subset of predicted-presence data with a normal-error model to the observed ($\ln(n + 0.1)$-transformed) counts using the same list of predictor variables noted above, and took our predicted values for each site and date from the second step in our analysis. The predicted counts were used to represent the latent (unmeasured but underlying) number of birds of each species visiting feeders at every site for each date on which the prevalence of house finches with conjunctivitis was reported.

We used logistic mixed models to identify relationships between latent abundances of each species and the probability that an observed house finch would have conjunctivitis at a site; the latent abundance of house finches was always included even when examining effects of the other two species' abundances. In addition to the latent counts of birds (previous paragraph), our models contained season, observation date during the season, a measure of observer effort, an interaction of season and house finch abundance as predictor variables, and latitude. Latitude was present in order to account for any gradient in disease prevalence (Dhondt et al. 2005) not related to local variation abundances of birds. We treated study site as a random effect. The effects of host species abundance were examined using five separate statistical models: a model containing latent abundance of only house finches, and separate models with latent abundances of each alternate species and their interaction with house finches. We used a p-value ≤ 0.05 to indicate statistical significance, and thus to determine which of these statistical models best fit our data. We found no

statistically-significant interactions between abundances of house finches and either of the other two species and will not discuss these two models further. The model with latent abundance of cardinals fit our data best (Table 6.1); we found that an increasing abundance of house finches and cardinals was associated with the highest disease prevalence (Figure 6.1). This supports our earlier conclusion (Hochachka and Dhondt, 2006) that higher abundances of house finches result in higher disease prevalence. Further, disease prevalence was highest when cardinal abundance was high,

Table 6.1. Generalized linear mixed model results for the effect of alternate host species abundance on house finch disease prevalence.

Effect	Numerator	HOFI[b]		NOCA[c]		AMGO[d]	
	df[a]	F	*P*	F	*P*	F	*P*
Latitude	1	4.47	**0.035**	5.61	**0.018**	3.01	0.083
Season	6	1.2	0.304	0.83	0.543	1.24	0.284
Half Day Effort	3	1.55	0.200	1.59	0.190	1.58	0.192
Date (3 wk period)	7	1.25	0.272	1.14	0.337	1.29	0.251
estimated HOFI	1	0.17	0.681	0.03	0.873	0.07	0.794
estHOFI*Season	6	3.52	**0.002**	3.43	**0.002**	3.37	**0.003**
estimated CARD	1	-	-	5.61	**0.018**	-	-
estimated AMGO	1	-	-	-	-	2.74	0.098

[a]denominator df: house finch only model 3826; other models 3825
[b]HOFI: model including house finch abundance only
[c]NOCA: model including house finch and northern cardinal abundance
[d]AMGO: model including house finch and American goldfinch abundance

suggesting that cardinals may be able to amplify disease risk in the primary host. While the potential exists for combinations of abundances of house finches and cardinals to result in higher disease prevalence in house finches, we rarely observed

sites with species' abundances that would yield the highest predicted disease prevalence (Figure 6.2). Less than 6% of sites had combinations of species'

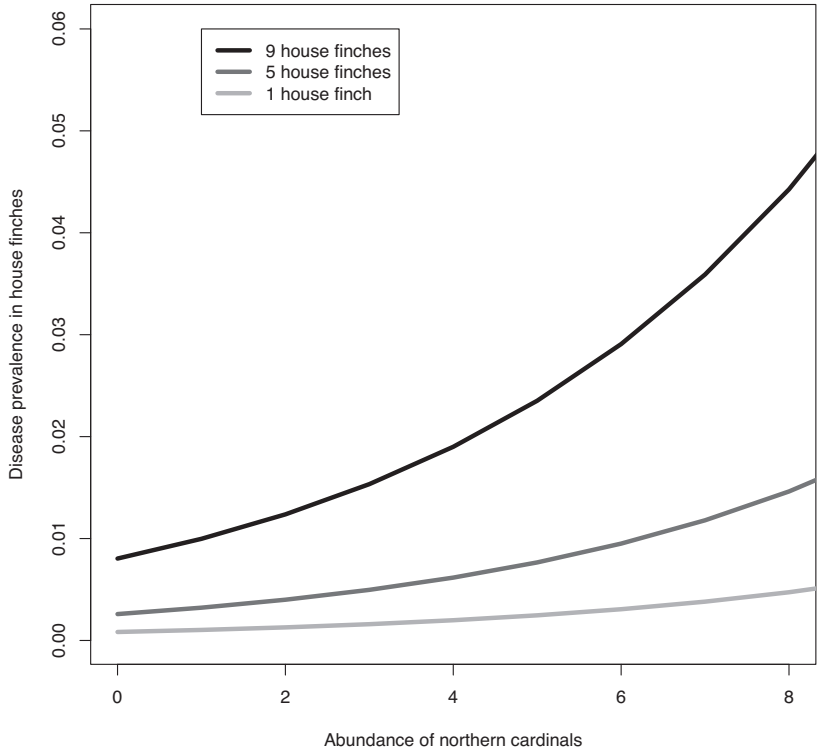

Fig. 6.1. Additive effect of the abundances of house finches and northern cardinals on disease prevalence in house finches. Previous work indicated that cardinals are a competent reservoir for *M. gallisepticum*. Darker lines represent an increasing abundance of house finches, and a larger abundance of house finches is associated with higher disease prevalence. Disease prevalence also increases with a higher abundance of cardinals. When both species are abundant, higher disease prevalence was observed: a possible amplification effect. All but the top 1% of house finch and cardinal counts are included in this figure.

abundances in which we would expect the upper 10% of predicted disease prevalence levels. We found that a higher latent abundance of American goldfinches was also associated with higher disease prevalence in house finches, although this effect was not marginally significant.

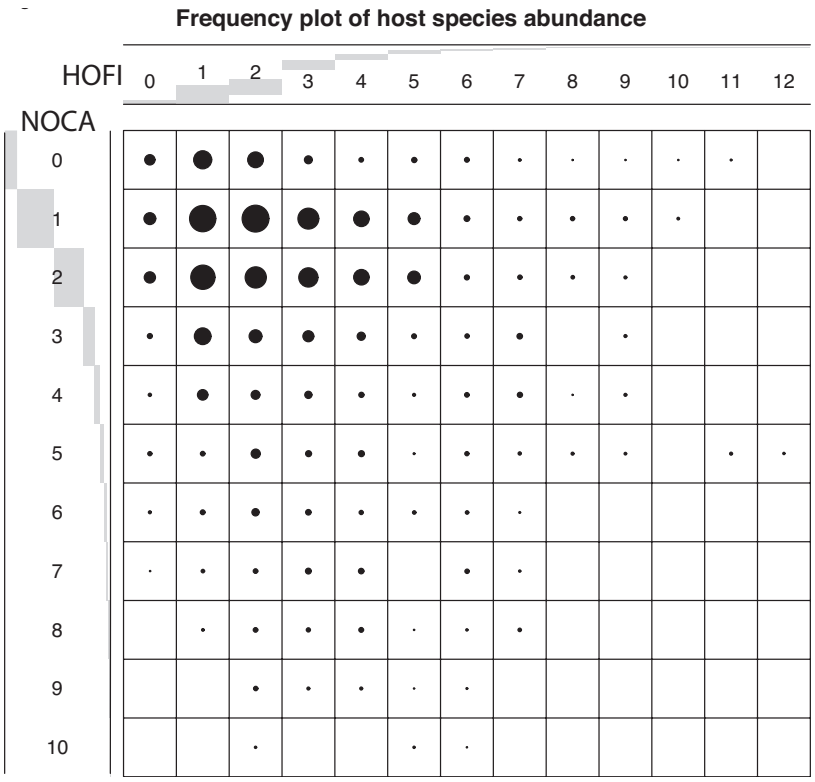

Figure 6.2. Frequency plot of combinations of house finch and northern cardinal abundances. The top and left axes represent house finch (HOFI) and northern cardinal (NOCA) counts, respectively. The sizes of the dots represent the number of sites at which a particular combination of the two host species was observed. Sites with high abundances of both species – where highest disease prevalence is expected – are uncommon.

With our demonstration that abundances of house finches and northern cardinals can affect disease prevalence in house finches, we expect that geographic variation in abundance of these two species will lead to geographic variation in prevalence of mycoplasmal conjunctivitis. Looking for spatial variation in abundances of the species, visually we found no evidence of spatial clustering of house finch or northern cardinal abundance (Figure 6.3). Additionally, in a formal analysis, no significant spatial autocorrelation was found at any inter-site distance for house finches, and only weak clustering at <40km for northern cardinals (Figure 6.4).

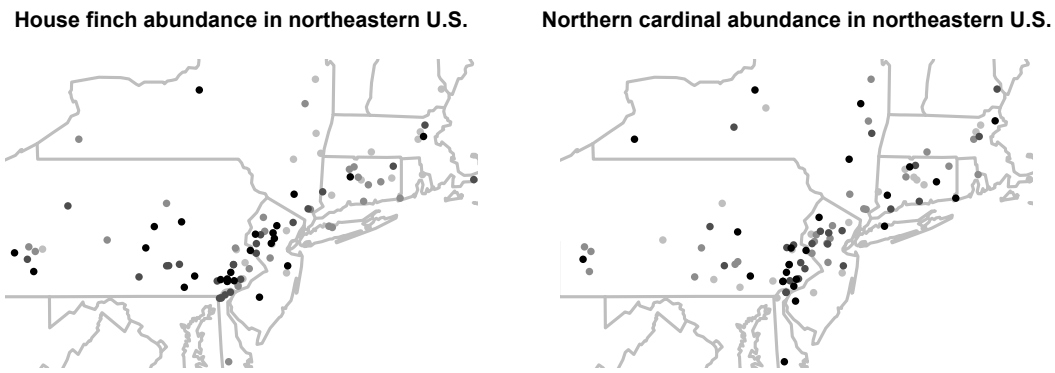

Figure 6.3. Maps indicating that both house finch (left) and northern cardinal (right) abundances varied from site to site throughout the study region. A dot represents the mean abundance for each host species across all observations at a specific site. Higher species abundances are represented by darker dot colors.

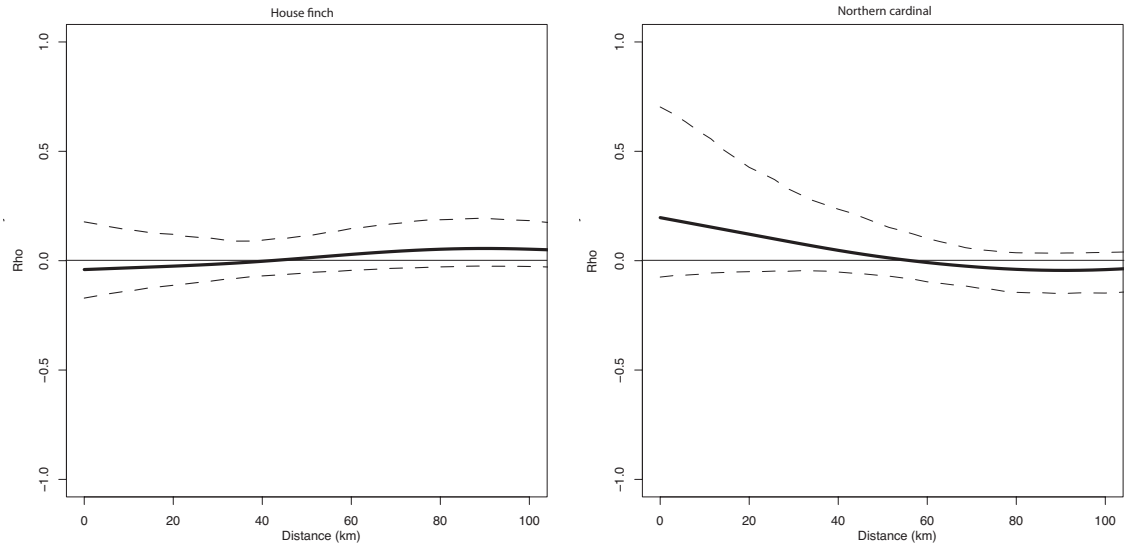

Figure 6.4. Evidence of no spatial clustering of house finch (left) and weak spatial clustering (right) of northern cardinal abundances at distances <100 km. Solid curves are spline correlograms, and bootstrapped ninety-five percent confidence limits (dashed lines) that do not contain the zero correlation line (horizontal line) indicate that abundances at nearby sites are more similar than would be expected by chance. Significant spatial autocorrelation was only seen for northern cardinals at inter-site distances of ≤40 km. Even here, the magnitude of autocorrelation was low.

Because host species' abundances have the potential to influence disease prevalence and sites with high and low abundances were interspersed throughout the study region, we expected similar variation in disease prevalence throughout the study region. Indeed we found that there was no detectable pattern to disease prevalence in house finches at a regional level (Figure 6.5), and spatial autocorrelation in prevalence of disease was effectively zero among all sites within our study area regardless of distances among sites (Figure 6.6).

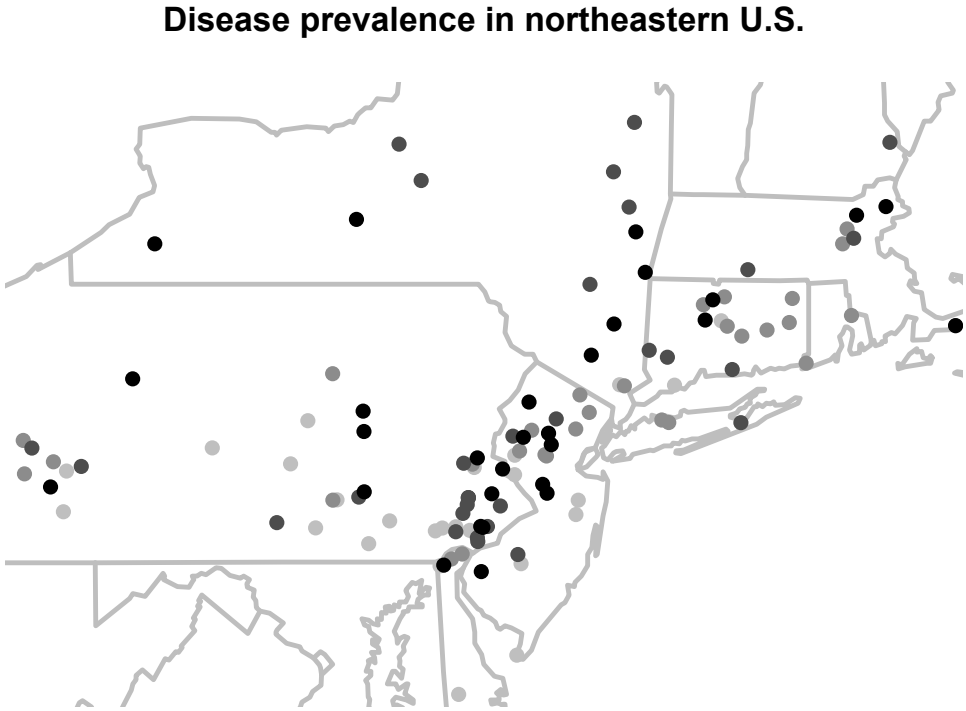

Figure 6.5. Variation in disease prevalence among sites throughout the study region. Like species abundances, there is a wide variation in disease prevalence even between nearby sites. Dots on the map are as in Figure 6.3.

In conclusion, alternate host species abundance has the potential to affect disease prevalence in house finches, although we rarely found the specific combinations of abundances that were associated with highest disease prevalence. Abundances of northern cardinals and house finches varied on a local scale; there is little to no correlation even between nearby sites. As with patterns of abundance, we saw no large-scale spatial structure in disease prevalence; rather, only site-to-site variation in levels of disease prevalence.

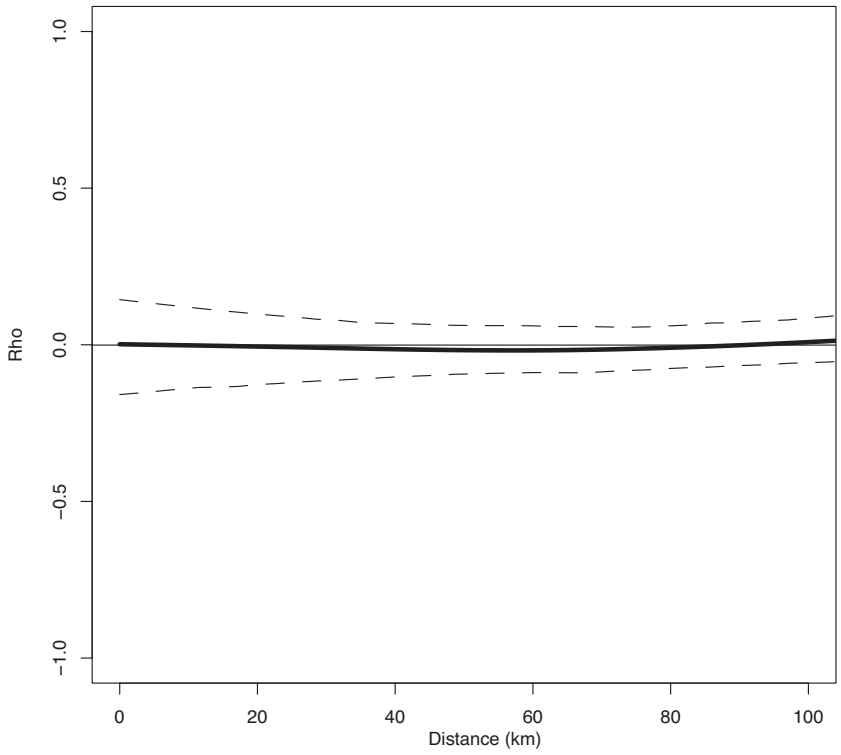

Figure 6.6. No spatial clustering of disease prevalence at distances less than 100 km. This indicates that high disease prevalence at one site was not associated with high disease prevalence at nearby sites. Lines on the graph are as in Figure 6.4.

When other species are competent hosts and reservoirs of disease, they have the potential to reduce the focal host population, which could be an important consideration for managing species of conservation concern. Many other avian species test positive for infections of *M. gallisepticum* in the field; we need a better understanding of the infection prevalence of these alternate hosts and the efficiency with which the pathogen is transmitted between these species and house finches. However, based on our findings of the effects of northern cardinal and American

goldfinch on disease prevalence in house finches, it appears the host-community effects on disease prevalence in house finches are subtle at best.

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CHAPTER 7

CONCLUSION

Variation at multiple levels of a host-pathogen system can significantly impact the outcomes of infection and disease within the host population. I have addressed a variety of questions regarding the risk of infection and likelihood of transmission of *M. gallisepticum* among individuals, within populations, and with the avian community, but many interesting questions still remain.

The work presented here builds on extensive work exploring variation in immunological and behavioral responses of house finches to *M. gallisepticum* infection (Hawley 2006, Hawley *et al.* 2006, 2007a, 2007b). I demonstrate that certain host physiological and behavioral characteristics can affect an individual's risk of infection and shape infection dynamics within a population. I found that behaviors associated with indirect transmission through feeders and dominance status were most important for predicting infection risk. I also found moderate support for increased infection risk when the source of infection was a highly social bird.

Although I was able to identify behaviors that were predictive of infection risk, further exploration of the mechanisms contributing most to direct transmission are necessary, since we cannot exclude that this mode is unimportant to *M. gallisepticum* spread. Finches are gregarious and social; however, I do not know the exact behaviors or physical contacts between birds that permit a transmission event. Although *M. gallisepticum* resides primarily in the conjunctiva, behavioral observations rarely, if ever, documented direct contact between birds' eyes. Perhaps *M. gallisepticum* can

reside on and be spread by contact with feathers or feet. We have also not determined the relative importance of physical contact that may occur during behaviors such as courtship, mating, or roosting. House finches share small, communal roosts, and infected individuals move more frequently between roosts (Dhondt *et al.* 2007), possibly distributing the pathogen, but we do not know the exact behaviors occurring at roosts. Roosting behavior is known to affect the spread of West Nile Virus within and among bird species (Ward *et al.* 2006). Perhaps specific behaviors involving direct interactions between individuals at roosts could contribute to pathogen spread.

Given that feeder-associated behavior appears to be important for *M. gallisepticum* transmission, there are still relevant questions regarding the exact mechanisms and relative importance of both indirect and direct transmission. Though I found no differences in viability over time between antimicrobial and standard feeders, and thus could not explicitly compare transmission modes, this remains a critical experiment in this system. In comparing pathogen viability in these antimicrobial feeder experiments, I found that *M. gallisepticum* is viable for a longer amount of time than previously determined. This necessitates a better understanding of both the threshold load for infection, and how pathogen load might interact with both transmission mode and host behavior. Do pathogen isolate differences influence the relative importance of transmission mode? For isolates with a higher threshold load for infection, perhaps the chance of infecting a host might depend more on the frequency of contact with infected hosts or fomites.

I have shown that high pathogen load and skewed distribution among hosts leads to higher rates of infection in host groups, suggesting that pathogen variation can

drive patterns in the rates of *M. gallisepticum* transmission at the population level. House finches are partially migratory (Belthoff and Gauthreaux 1991), and dispersal of high pathogen load individuals could be responsible for regional spread of *M. gallisepticum*. The opportunity for pathogen transmission is likely even greater if pathogen load peaks prior to the onset of disease symptoms that could reduce host movement and survival. Short-distance transmission of high pathogen load isolates of *M. gallisepticum*, if successfully transmitted among hosts, might allow the evolution of increased virulence. There is already evidence of isolate-based differences in virulence that likely facilitated long-distance movement of *M. gallisepticum* from the East to the West coast (Hawley *et al.* 2010). We need a better understanding of the relationship between pathogen load and virulence.

I provide evidence that asymptomatic adults can infect naive juveniles, and this phenomenon likely drives the seasonal patterns of disease prevalence in wild populations. I also show that the introduction of an infected individual into a population can initiate a new epidemic with a host group. These patterns of group infection dynamics necessitate exploration of individual stress and immune responses that occur at higher host densities, which might influence population-level patterns of infection and disease. We could ask how these responses influence: 1) whether a bird becomes infected, 2) whether an individual becomes diseased, and 3) the severity of infection and time to recovery. A longitudinal study of immunological changes may help explain patterns of infection within these groups. We also could ask whether superspreaders, individuals characterized by either high host contact frequency or pathogen load, are necessary to generate new epidemics and whether there are

differences in the pathogen threshold necessary for generating epidemics within susceptible versus recovered groups.

Many other passerine species are susceptible to *M. gallisepticum* infection, while only a small subset of these species develops clinical signs (Hartup *et al.* 2000, Mikaelian *et al.* 2001, Farmer *et al.* 2005). Previous work documented that house sparrows and American goldfinches can infect house finches, with the latter being more infectious (Dhondt *et al*. 2008). My work found that northern cardinals increased the prevalence of *M. gallisepticum* in house finches when both species were present at a site. We need a better understanding of the mechanisms by which MG is maintained and transmitted among other host species that drive higher prevalence patterns within house finches. These questions require an understanding of both immunological and behavioral factors, as both could affect infection prevalence within the host community. Furthermore, what are the immunological differences that make house finches more susceptible and likely to develop severe eye lesions?

This work adds to the understanding that heterogeneities at multiple levels of a host-pathogen system can both cause variation in the individual risk of infection and determine the rate of pathogen transmission within the host population. I have shown that variation intrinsic to the host, pathogen, and environment can influence the likelihood and patterns of *M. gallisepticum* among house finches. Rarely are host populations simply homogeneous, randomly-mixing groups; when possible, we must account for this potentially wide and dynamic variation present in these host-pathogen systems to better understand infection or disease dynamics over time.

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APPENDIX A

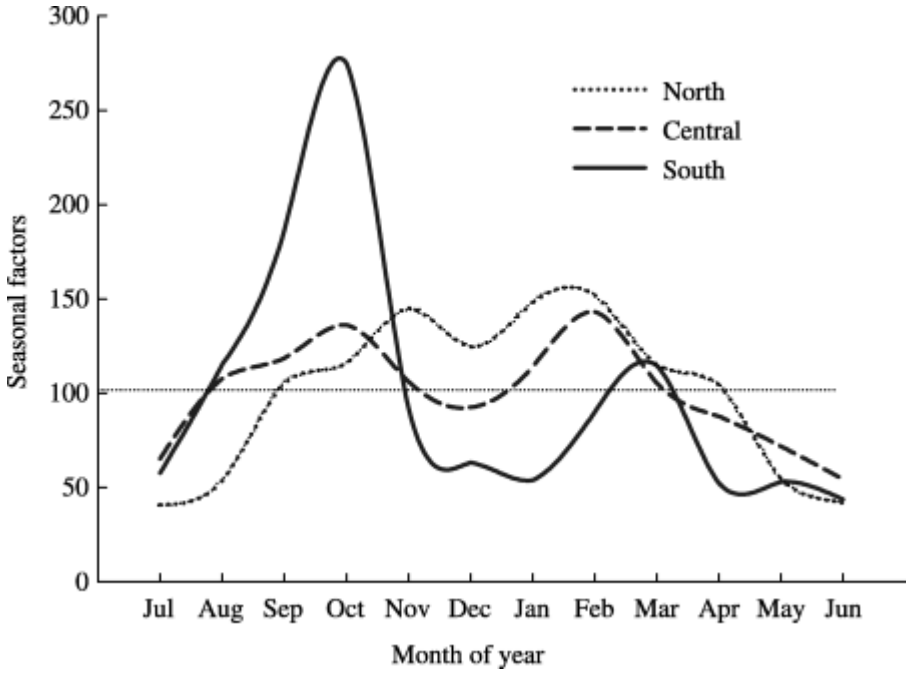

Figure A.1. Seasonal variation in prevalence of mycoplasmal conjunctivitis in three regions illustrating the bimodal variation: peaks are reached in late summer/ fall, and in late February/early March, while minimal values are observed in December and during the breeding season (from Altizer et al. 2004) .

Figure A.2. A picture of the large aviary systems used in this experiment. Each flock of birds had access to two octagonal cages connected by a corridor.