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GENEALOGICAL RELATIONSHIPS INFLUENCE THE PROBABILITY OF INFECTION WITH BOVINE TUBERCULOSIS AND MICROGEOGRAPHIC GENETIC STRUCTURE IN FREE-RANGING WHITE-TAILED DEER

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ABSTRACT

GENEALOGICAL RELATIONSHIPS INFLUENCE
THE PROBABILITY OF INFECTION WITH BOVINE TUBERCULOSIS
AND MICROGEOGRAPHIC GENETIC STRUCTURE
IN FREE-RANGING WHITE-TAILED DEER

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Zoonoses are of increasing importance to wildlife conservation and human health. It has become increasingly recognized that wildlife ecology plays a key role in disease transmission in wildlife populations. In domestic populations, contacts among individuals are controlled by humans and disease transmission is often density dependent. Unlike domestic animals, wildlife populations often have complex social systems in which contacts among individuals are not solely density dependent, and can play an important role in the transmission and maintenance of disease.

White-tailed deer (*Odocoileus virginianus*) in the northeast lower peninsula of Michigan (MI) are infected with bovine tuberculosis (*Mycobacterium bovis*) (TB). The practice of artificial feeding that brings large numbers of deer into contact likely facilitated the transmission of TB. Artificial feeding was banned in order to reduce contacts among individuals at artificial feeding sites and thereby reduce the risk of TB infection. White-tailed deer ecology may also play an important role in the probability of infection with TB. Deer have a complex social system in which females live in related groups (matrilines). The rate of contact among individuals within matrilines (relatives) is high relative to contact rates among individuals from different matrilines. Estimates of genealogical relationships were used to infer the role that white-tailed deer's social

structure played in the risk of TB infection. TB-infected deer were significantly more closely related than were non-infected deer. Relatedness between pairs of TB-positive deer was higher than relatedness between non-infected deer independent of the occurrence of artificial feeding. The probability of TB infection was not random, but rather a function of genetic relatedness to TB-infected individuals. It can be inferred from these data that matrilines of deer likely serve as reservoirs of TB within the deer population.

White-tailed deer's matrilineal social structure would be expected to result in heterogeneity in allele frequencies among matrilines across space. Artificial feeding of the deer population in MI, however, results in the congregation of large numbers of individuals at artificial feeding sites. Molecular markers were used to characterize the impact of artificial feeding on deer spatial genetic structure in the northeast lower peninsula of MI. Spatial autocorrelation analyses indicated that when artificial feeding was occurring there was no significant relationship between the degree to which quadrats of deer were genetically differentiated and their geographic distance from one another. The aggregation of multiple matrilines at feeding sites likely homogenized spatial genetic structure. Following the ban on artificial feeding, there was significant heterogeneity in allele frequencies among groups of deer as a function of geographic distance. The significant microgeographic genetic structure that exists within the deer population following the ban on artificial feeding indicates that transmission of TB across genetically differentiated groups is likely to be limited.

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CHAPTER 1

INTRODUCTION

Historical background of white-tailed deer and bovine tuberculosis in Michigan

The white-tailed deer (*Odocoileus virginianus*) is the oldest deer species in North America, first appearing in the late Pliocene before the glaciations of the Pleistocene (Geist et al. 2000). Prior to European settlement, populations of white-tailed deer were widespread throughout what is currently the United States, and may have approached 26 million animals (Geist et al. 2000). In Michigan (MI), before human settlement, white-tailed deer were most abundant in the southern part of the state where the habitat primarily consisted of open oak and hickory forests ideal for deer (Langenau 1994, Michigan Department of Natural Resources (MDNR) 1993). In the northern lower peninsula of MI, forests were primarily pine and hardwood that did not provide optimal habitat for deer (MDNR 1993). When settlers first arrived in MI in the 1850s, their clearing of land through logging and burning initially increased deer habitat quality (Langenau 1994, MDNR 1993). However, as settlement progressed and the human population grew larger, over-clearing, and burning of forests increased the openness of the land and decreased its quality for deer (Langenau 1994). In addition, increased numbers of settlers resulted in an over-harvest of the deer population (Langenau 1994). In the 1870s, the deer population in MI was estimated to be one million and had dramatically declined in southern MI as the result of decreased habitat quality and excessive commercial harvest (Langenau 1994, MDNR 1993). Most deer in the late

1870s were found in the northern lower peninsula where white pine logging created openings in the forest resembling habitat found in presettlement southern MI (MDNR 1993). At the end of the 19th century, the deer population in MI had declined to fewer than 500 thousand due to heavy market hunting and uncontrolled logging (MDNR 1993). Extensive fires on land after logging slowed the recovery of forests, and deer were not able to survive in open grasslands that did not provide sufficient food or winter cover (MDNR 1993).

During the early 20th century, the Lacy Act ending commercial deer harvest together with Pittman-Robertson funds for habitat restoration allowed the deer herd in MI to increase to about 1.5 million animals by 1949 (MDNR 1993). Most of these deer were found in the northern lower peninsula where habitat was best and the human population was smaller than in the southern part of the state (MDNR 1993). However, deer habitat quality declined again in the 1950s as second growth forest matured leaving few openings and poor food resources (MDNR 1993). As a result of habitat decline and an increase in antlerless hunting, the deer population declined in MI such that by 1972, only approximately 85 thousand deer remained in southern MI with fewer than 500 thousand in the entire state (MDNR 1993). In recent years, an increase in the timber industry along with mild winters and supplemental feeding has allowed the deer population to increase (MDNR 1993). By the mid-1990s deer numbers neared 2 million and densities in northeastern MI averaged >35/ km^2 (Schmitt et al. 1997, Hickling 2002).

Carrying capacity for white-tailed deer in forested habitats in the northeast lower peninsula of MI was historically low. During the early 20th century, numerous hunting clubs were established in northeast MI. Due to high hunting demand, shortage of natural

browse was alleviated through artificial feeding to enhance deer population numbers. Artificially high deer densities resulting from artificial feeding practices has the potential to cause damage to the natural vegetation and to have negative effects on the deer population itself by increasing the transmission and prevalence of disease (Chouinard and Filion 2001, Miller et al. 2003).

Currently, the white-tailed deer population in MI, primarily in the northeast lower peninsula, is infected with bovine tuberculosis (*Mycobacterium bovis*) (TB) (Figure 1). This is the only occurrence of self-sustaining bovine TB in a free-ranging cervid population in North America. There are no well-established control programs for TB in wild deer, and at the time of its discovery in MI, much about the transmission of bovine TB was unknown. TB has also been discovered in cattle herds (dairy and beef) and other wildlife (bears (*Ursus americanus*), bobcats (*Lynx rufus*), coyotes (*Canis latrans*), elk (*Cervus elaphus*), opossums (*Didelphus virginiana*), raccoons (*Procyon lotor*), and red foxes (*Vulpes vulpes*)) within the state. As a result of the presence of TB in livestock, the state's TB-status was downgraded from "accredited-free" to "modified accredited-free" (USDA 2000). The loss of "accredited-free" status has resulted in movement restrictions and mandatory TB testing for livestock. The costs of the imposed regulations on the beef and dairy industry, TB testing of wildlife and livestock, and compensation for depopulated herds has made Michigan's TB problem a multimillion dollar issue. There is a collaborative research effort among wildlife biologists, veterinarians, microbiologists, epidemiologists, and managers to understand and control bovine tuberculosis in free-ranging wildlife and domestic animals. Research indicates that white-tailed deer are the reservoir host for TB in MI while other wildlife are hypothesized to be only spillover or

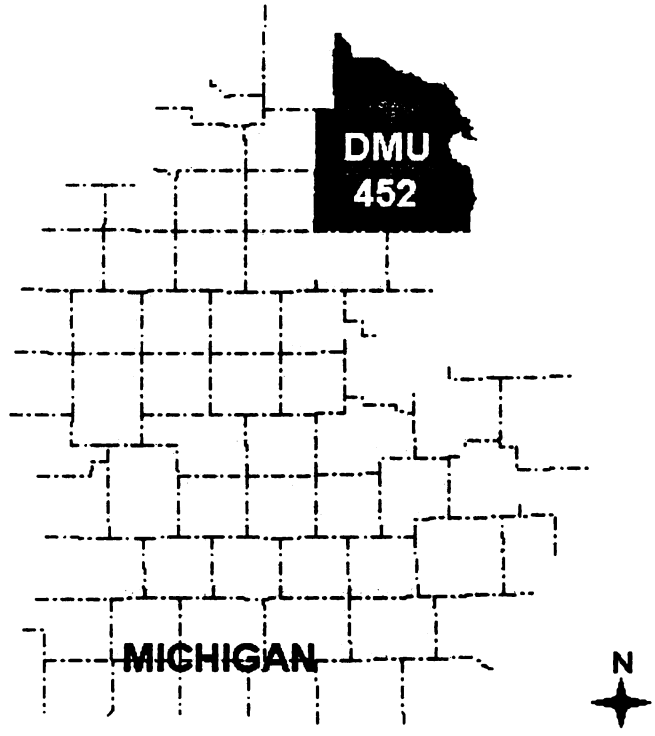

Figure 1. Map of the lower peninsula of Michigan highlighting the 5 county region where the deer population is infected with bovine tuberculosis (DMU452).

dead end hosts (Schmitt et al. 1997). One of the goals in studying TB in MI, as stated by the United States Department of Agriculture (USDA), is to determine the long-term, baseline prevalence of TB and the rate of TB transmission in white-tailed deer.

During the early 20th century, bovine TB was found in high prevalence within cattle populations throughout MI. As the result of an intensive eradication program, MI livestock gained USDA TB-free accreditation in 1979 (MDNR Activity Report 1997). However, in 1975, a TB-infected deer was harvested in Alcona county and a second TB-positive deer was identified in Alpena county in 1994 (Schmitt et al. 1997). In 1995, the Michigan Department of Natural Resources (MDNR) screened livestock and hunter-harvested deer for TB in the counties surrounding the region where the first 2 TB-positive deer were harvested. While no infected livestock were found to be infected at that time, an additional 18 TB-infected deer were identified (Table 1). The 5 counties from which TB-infected deer were harvested in the northeast lower peninsula of MI were designated the main TB management area, named Deer Management Unit 452 (DMU452), and included Alcona, Alpena, Montmorency, Oscoda, and Presque Isle counties. The area in the northeast lower peninsula of MI designated DMU452 has changed over time. For the purposes of this dissertation, DMU452 will refer to the 5 county area that DMU452 encompassed at the beginning of this study. From 1995 to the present, hunters were asked to voluntarily submit the heads of deer harvested from throughout MI to MDNR check stations. Deer heads were sent to the MI Animal Health Diagnostic Laboratory where the cranial lymph nodes were visually inspected for signs of infection with TB. Suspected infections were cultured and serially tested with a genetic probe for *M. bovis* serving as the definitive test. The MDNR's survey resulted in the identification of an additional 336

Table 1. Number of harvested deer screened for TB and number of TB-positive deer in Michigan from 1995-2000.

Year	Deer Screened	TB-positive Deer
1995	403	18
1996	4967	56
1997	3720	73
1998	9057	78
1999	19500	58
2000	25858	53

TB-positive deer in MI from 1995-2000 (Table 1) (O'Brien et al. 2002). In addition to increased surveillance of wildlife for TB, management is aimed at eradication of TB from the deer population in order to eliminate the spillover of TB into livestock.

Models of disease transmission

Interactions between host and pathogen populations are driven by transmission dynamics (Begon et al. 1999). Host-pathogen models are used to design management strategies for disease control (McCallum et al. 2001). Traditionally, in the majority of both empirical and theoretical studies, disease transmission dynamics have been assumed to be density dependent (mass action) (Anderson and May 1992, Grenfell and Dobson 1995, Begon et al. 1999, McCallum et al. 2001). In density dependent models of disease transmission, contacts among infected and susceptible individuals are assumed to be random and to increase in proportion to population size (Begon et al. 1999). Culling in order to reduce population density is a common management strategy for controlling disease based on density dependent models (McCallum et al. 2001). This policy will fail, however, if disease transmission is not host-density dependent (McCallum et al. 2001).

Alternatively, disease transmission dynamics may be frequency dependent where contacts among infected and susceptible individuals are independent of population size (Begon et al. 1999). In addition, incidence of infection is often patchily distributed, where infected individuals are likely to be closer to other infected individuals than expected under random mixing, the typically assumed model of disease transmission (McCallum et al. 2001). The manner in which transmission dynamics are modeled will have profound

effects on the success of management actions aimed at eliminating disease transmission (Dobson and Meagher 1996).

Most zoonotic pathogens are endemic, always present at some low level, in wildlife populations (Childs et al. 1998, Begon et al. 1999). Most empirical studies of disease transmission in wildlife, however, have been conducted opportunistically in response to epidemic outbreaks which generally occur over short time periods (Begon et al. 1999). Endemic diseases are rarely easily eradiated, and ecological changes can promote their amplification to epidemic levels (increased incidence) (Childs et al. 1998). As such, it is important to understand the key mechanisms of disease transmission to devise effective control measures (Childs et al. 1998).

Recently, numerous studies have focused on determining the mechanisms of disease transmission in natural populations. Boone et al. (1998) evaluated the ecological correlates of hantavirus in deer mice. They found a complex non-linear relationship between density and disease prevalence such that above a certain threshold, density of deer mice no longer influenced hantavirus prevalence (Boone et al. 1998). In addition, there are often differences in the frequency of infection among demographic segments of wildlife populations (i.e. age, sex) (Mills and Childs 1998). Patterns of cowpox infection in rodents vary as a function of age and sex (Hazel et al. 2000). The difference between male and female tick dispersal and host specificity may be important in understanding Lyme disease transmission (Meeus et al. 2002). Higher rates of Puumala virus infection among adult male bank voles in comparison to the rest of the population was used to infer that aggressive interaction among males was a major mechanism of disease transmission (Escutenaire et al. 2002).

The management actions taken to eliminate TB from free-ranging deer have been built upon a density dependent model of epidemic disease transmission. TB management strategies focus on actions aimed at minimizing social contact and crowding of deer at artificial feeding sites and on decreasing population numbers. One major action taken to decrease the transmission of TB among deer was to ban both supplemental feeding and baiting in DMU452. Supplemental feeding is defined as the placement of food designed to help deer survive harsh winters. Baiting is defined as putting food out for the specific purpose of attracting deer for hunting. The basis for the bans was the result of an in-depth risk assessment conducted on the TB problem in MI by the USDA that recommended decreasing interactions among individuals and increasing hunting to decrease the population size (MDNR TB Activity Report 1997). Artificial feeding brings large numbers of deer into contact for prolonged periods of time, in contrast to the normal feeding practices of deer where they remain spread out in space foraging over greater distances in order to obtain necessary resources. At artificial feed sites, the probability of encountering food contaminated with *M. bovis* is greater than in a naturally feeding deer population due to the large numbers of deer that feed at supplemental sites (MDNR Activity Report 1997). In the absence of artificial feeding sites, it was hypothesized that deer would disperse in space in search of food and would not congregate at feeding sites. It was believed that the elimination of large aggregations of deer would decrease TB transmission.

The second major management action was to increase the antlerless (doe) deer harvest in DMU452 in order to decrease overall deer population density. Increased hunting, especially of female deer, was intended to reduce deer numbers in DMU452 to

levels that could be supported by natural habitat. In addition, hunters from DMU452 were asked to voluntarily submit the heads of their harvested deer to the MDNR to check for signs of TB infection. It was hypothesized that the combination of both management strategies would reduce the transmission of bovine TB. When transmission decreases below a certain threshold, more deer infected with bovine TB will die each year than would become infected, and prevalence rate will decline.

The goal of management is to decrease disease prevalence to less than 1 percent by the fall of 2003, and to have the disease eliminated in the wild deer herd by the fall of 2010 (MDNR TB Activity Report 1997). The bans on supplemental feeding and baiting along with increased hunting are likely to result in decreased transmission of TB among the deer population as a whole. However, because of the behavior and social structure of white-tailed deer populations that results in nonrandom interactions among individuals (Hawkins and Klimstra 1970, Marchinton and Hirth 1984), the feed ban and reduction in population density alone are not likely to eradicate TB. An understanding of the role of deer ecology in disease transmission is necessary to evaluate transmission dynamics between deer and *M. bovis* and whether current management actions will eliminate disease transmission.

It has become increasingly recognized that wildlife ecology plays a key role in disease transmission (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). In order to eliminate disease from wildlife populations it is necessary to understand host species' ecology. The preferred habitats of white-tailed deer are primarily deciduous and coniferous forests that provide cover (Marchinton and Hirth 1984). Males generally have larger ranges than females and

both sexes tend to use the same ranges across years (Marchinton and Hirth 1984) likely restricting the range over which an infected deer could transmit TB. Deer do not tend to migrate long distances, but do often make seasonal movements in search of food (Marchinton and Hirth 1984). The availability of preferred habitat and food types are likely to influence the distance and direction of deer movement, which in turn is likely to play a key role in the spread of TB geographically.

In addition to the influence of habitat preference and movement ecology, social structure and behavior are likely to play important roles in the transmission of disease in wildlife populations (Mills and Childs 1998, Begon et al. 1999). The social structure of white-tailed deer is likely to influence which animals are most likely to become infected with TB in the absence of artificial feeding. White-tailed deer have a matriarchal social structure (Hawkins and Klimstra 1970). Females live in groups usually consisting of an adult doe, her yearlings and fawns, and often her older offspring as well as offspring of female relatives. In a study of white-tailed deer in southern Illinois, Hawkins and Klimstra (1970) found that 87% of yearling females remained philopatric and were observed with other group members in greater than 60% of all observations. In contrast, 80% of yearling males were found to disperse (Hawkins and Klimstra 1970). For those males that did not disperse, they moved an average of 3 miles from their mothers' home ranges (Hawkins and Klimstra 1970). Adult and young males are occasionally found in groups of 2-5 during the non-breeding season. However, during the breeding season (September through December in MI) adult males are solitary. The strong site fidelity and frequency of contact among related females and male fawns suggests that matriarchal structure is likely a strong contributor to disease transmission. Specifically, the

probability of infection with TB is likely non-random such that deer that are closely related to an infected deer have a higher risk of TB infection than do nonrelatives.

Utility of molecular markers for studying disease in wildlife populations

The importance of a species' ecology in disease transmission is well documented. Many recent studies were conducted over multiple years in intensive field situations in which rates of infection differed as a function of age and sex (Boone et al. 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002). Differences in infection rate across segments of the population (i.e. age, sex) were used to infer how behavior influenced disease transmission. Long-term, field intensive studies are very difficult, time intensive, and expensive. Multi-year studies are often not practical for wildlife diseases that have reached epidemic levels and demand immediate management actions.

In order to evaluate the role of social structure in TB transmission, it is necessary to determine relationships among both TB-infected and non-infected deer. It would be impossible to directly observe the movement, behavior, and relationships of the entire white-tailed deer population in DMU452. In the absence of known pedigree relationships, molecular markers can be used to estimate genealogical relationships among animals (Avise 1994, Queller and Goodnight 1989, Lynch and Ritland 1999). Microsatellite markers are especially valuable for determining relationships among animals. Loci that possess large numbers of alleles as well as rare alleles are useful in determining genealogical relationships among individuals in close proximity (Parker et al. 1998). When individuals share an allele at a highly polymorphic locus there is an increased

probability that these alleles are identical by descent. The probability that alleles are identical by descent increases when individuals share alleles that are rare in the population. Multiple genetic markers can be used to estimate the degree of coancestry among individuals. Coancestry among individuals can be used to recreate pedigrees when direct observation of behavior is not possible and pedigrees are not known. Levels of coancestry among infected individuals when compared to coancestry among non-infected deer can be used to make inferences about mechanisms of disease transmission and the probability of infection within wildlife populations.

Analyses of wildlife spatial genetic structure can be used to infer the impact of management actions on movement patterns and breeding behaviors that would be difficult to determine using other methods. Population genetic structuring and gene flow can be measured indirectly by assaying geographic variation in the frequency of heritable genetic markers (Scribner et al. 1997). In order to characterize the pattern of genetic variability over large geographic areas, moderately variable microsatellite loci are desirable to characterize individuals within a population and distinguish them individuals from other populations (Parker et al. 1998). The change in deer spatial structure as measured by spatial variation in gene frequencies can be incorporated into management strategies aimed at delineating hotspots of TB-infected deer, targeting management actions, and predicting the transmission and distribution of bovine tuberculosis.

In this dissertation, genealogical relationships among deer are estimated to infer the role of white-tailed deer social structure and behavior in disease transmission in DMU452, and the impacts of management on deer spatial genetic structure. Results are incorporated into a simple model that highlights the role of deer ecology in disease

transmission dynamics. This is the first study of its kind to use genetics to infer the role that wildlife species' ecology plays, specifically in terms of behavior and social structure, in the transmission dynamics between host and pathogen populations.

Sample collection and general methods

Muscle tissue samples from the neck region of approximately 9000, 17,000, and 22,000 hunter-harvested deer submitted to the MDNR in 1998, 1999, and 2000 respectively, were collected. Deer were individually identified with unique tag numbers that corresponded to an MDNR database containing the sex, age, harvest location, and TB status of each animal. Each sample was placed in a 1.5 μL tube filled with a preservative tissue storage buffer or 95% ethanol, and labeled with the deer's tag number. Samples were stored frozen at either -20 or -70 °C. DNA was extracted from the muscle tissue of animals used in the analyses in the following chapters (~5000) using the QIAGEN DNEasy extraction kit and quantified using fluorometry or spectrophotometry. Extracted DNA was used for microsatellite amplification. Forty-three fluorescently labeled microsatellite markers were screened and over 30 loci were optimized using a subset of deer tissue from DMU452 (~ 100 animals) (Appendix I, II). Microsatellite regions were amplified using the polymerase chain reaction, separated using polyacrylamide gel electrophoresis, and visualized using a Hitachi FMBIO-II laser scanner. The markers and techniques described above were used to address the research questions presented in the following chapters.

CHAPTER 2

GENEALOGICAL RELATIONSHIPS INFLUENCE THE PROBABILITY OF INFECTION WITH BOVINE TUBERCULOSIS IN FREE-RANGING WHITE-TAILED DEER

Zoonoses are of increasing importance to wildlife conservation and human health (Childs et al. 1998). An understanding of the underlying mechanisms of disease transmission is necessary to control wildlife disease. Traditionally, in the majority of both empirical and theoretical studies, disease transmission dynamics have been assumed to be density dependent (Anderson and May 1992, Grenfell and Dobson 1995, Begon et al. 1999), where contacts among infected and susceptible individuals are assumed to be random and to increase in proportion to population size (Begon et al. 1999). Alternatively, disease transmission dynamics may be frequency dependent where the number of contacts among infected and susceptible individuals is fixed and independent of population size (Begon et al. 1999). The manner in which transmission dynamics are modeled will have profound effects on the success of management actions aimed at eliminating disease transmission.

The white-tailed deer (*Odocoileus virginianus*) population in Michigan (MI), primarily within a 5 county area of the northeast lower peninsula of MI (DMU452) is infected with bovine tuberculosis (*Mycobacterium bovis*) (TB) (Figure 2, Table 2). Direct contact between animals in which fluids are exchanged, or the ingestion of food previously contacted by a TB-positive animal are hypothesized to be the primary routes of TB transmission (Schmitt et al. 1997). Management actions taken by the Michigan Department of Natural Resources (MDNR) to eradicate TB are based upon a density dependent model of epidemic disease transmission. One of the primary management

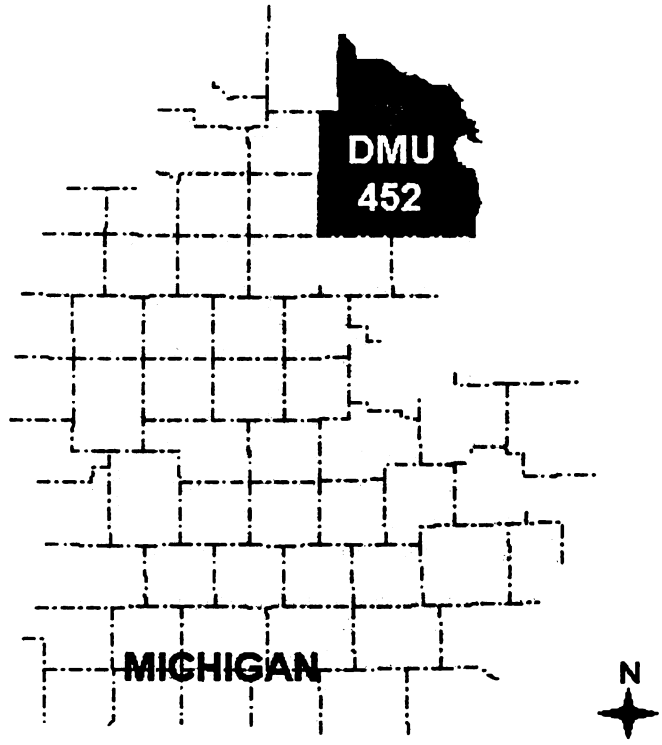

Figure 2. Map of the lower peninsula of Michigan highlighting the 5 county region where the deer population is infected with bovine tuberculosis (DMU452).

Table 2. Number of harvested deer screened for TB and number of TB-positive deer in Michigan from 1995-2000.

Year	Deer Screened	TB-positive Deer
1995	403	18
1996	4967	56
1997	3720	73
1998	9057	78
1999	19500	58
2000	25858	53

actions taken by the MDNR was to ban artificial feeding in 1999 in order to minimize contacts and crowding among deer. In addition, antlerless (doe) harvest levels were increased in DMU452 in order to decrease deer population size. Culling in order to reduce population density is a common management strategy for controlling disease based on density dependent models of disease transmission (McCallum et al. 2001). This policy will fail, however, if disease transmission is not host-density dependent (McCallum et al. 2001). The ban on artificial feeding and increased hunting do not account for aspects of deer ecology and social structure that may play important density independent roles in the transmission of disease. The ban on artificial feeding and the reduction of population density alone, therefore, may not eradicate TB from the deer population.

In order to understand and eliminate disease in a wildlife population it is necessary to understand characteristics of the species' ecology that might play important roles in the transmission and maintenance of disease (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). Complex social systems and behavior are likely to play important roles in the transmission of disease in wildlife populations. Aspects of white-tailed deer social structure and behavior are likely to play important roles in TB transmission. Deer have a matriarchal social structure in which females live in groups usually consisting of an adult female, her yearlings and fawns and often her older offspring, her sisters and their offspring (Hawkins and Klimstra 1970). Hawkins and Klimstra (1970) found that 87% of yearling females remained philopatric and were observed with other group members in greater than 60% of all observations. In contrast to the strong association among female

deer, 80% of yearling males were found to disperse (Hawkins and Klimstra 1970). For those males that did not disperse, their main areas of use were on average 3 miles distant from their mothers' home ranges (Hawkins and Klimstra 1970). Adult and young males are occasionally found in transitory groups of 2-5 during the non-breeding season (Marchinton and Hirth 1984). However, during the breeding season, which runs from September through December in MI, adult males are solitary (Marchinton and Hirth 1984). The strong site fidelity and frequency of contact among related females indicates that the matriarchal structure is likely to facilitate disease transmission within matrilines relative to the probability of transmission across matrilines. Consideration of the influences of movement and social structure on TB transmission is important when devising management strategies to eradicate TB from the deer population.

The high degree of social structure present in most white-tailed deer populations suggests that another likely risk factor for TB transmission that has not been accounted for in current MDNR management actions is genetic relatedness to TB-infected deer. If genetic relatedness plays a role in the probability of infection with TB, those deer at greatest risk for TB infection will be members of family groups in which one or more members is already infected. In the absence of artificial feeding, deer will be forced to spread out over space in search of food and will not crowd together at feeding sites. Following the feeding ban, deer coming into closest, most frequent contact will be close relatives. Thus, it was hypothesized that those deer most likely to become infected with TB would be close relatives of TB-infected deer. It was hypothesized that artificial feeding served to exaggerate TB transmission by bringing many different family groups into contact at feed sites. Transmission at artificial feeding sites facilitated the movement

of TB from within a few lineages to across many (and likely uninfected) genealogical lineages. In the absence of artificial feeding, congregations of large numbers of unrelated deer at feeding sites should decrease and TB transmission among non-relatives should be less frequent. However, if genetic relationships play an important role in the probability of TB infection, elimination of artificial feeding alone will not eliminate disease transmission among deer.

Determination of all mechanisms of TB transmission is important for devising management actions that are likely to successfully control the disease (Childs et al. 1998). If genealogical relationships prove to be important risk factor for TB infection, the disease is likely to be endemic, persisting within some matrilines. As a result, additional management strategies beyond the ban on artificial feeding and current level of population density reduction would be necessary to suppress transmission. In order to determine the importance of deer ecology, specifically genealogical relationships, in TB transmission, relationships among both TB-infected and non-infected deer in DMU452 needed to be determined. It would be impossible to directly observe the movement, behavior, and relationships of the entire white-tailed deer population in DMU452. In the absence of known pedigree relationships, molecular markers can be used to estimate genealogical relationships among animals. If TB transmission is non-random with respect to genealogical relationships, and if social structure plays an important role in the probability of infection with TB, it was hypothesized that, on average, TB-positive deer would be more highly related than non-infected deer. In particular, if the white-tailed deer's ecology itself plays an important role in the transmission and maintenance of TB,

we expected that TB-positive deer would be more highly related than non-infected deer independent of the presence or absence of artificial feeding.

Methods

Muscle tissue samples were collected from the neck region of hunter-harvested deer submitted from throughout the state to the MDNR during October-December of 1998, 1999, and 2000. Sample storage and DNA extraction were carried out as described in Chapter 1. All TB-positive deer harvested in DMU452 during 1998-2000 and whose harvest location was identified to 2.6-km^2 were selected for genotyping. The number of harvested TB-positive deer each year was only a small fraction of the total number of deer harvested and screened for TB (Table 2). In order to more accurately characterize allele frequencies in the deer population in DMU452, numerous non-infected deer were also genotyped (Table 3). Non-infected samples were chosen based on research on the movement patterns of deer in DMU452 that indicated the average maximum movement of deer was approximately 12 kilometers (Garner 2001). For each TB-positive deer, a subset of non-infected deer within the 12 kilometer radius were also identified and selected for genotyping.

All samples were genotyped at 11 polymorphic microsatellite loci (BL42, BM4107, Cervid 1, Cervid 2, ETH152, OarFCB193, RT 20, RT 23, RT 24, RT 27, and SRCRSP-10; Appendix I, II) (Buchanan and Crawford 1993, Bhebhe et al. 1994, DeWoody et al. 1995, Talbot et al. 1996, Wilson et al. 1997). Precise estimation of relatedness requires negligible genotyping errors, no null alleles, selectively neutral alleles, a rarity of mutations, random mating, and independent/ unlinked loci (Van de

Table 3. Sampling area and number of deer used in the estimation of genealogical relationships among TB-positive deer (TB+) and among non-infected deer (TB-) from 1998-2000.

Year	Sampling Area	TB+	TB-
1998	30.1 x 43.2 mi	67	547
1999	21.6 x 33.0 mi	46	311
2000	51.6 x 51.9 mi	35	253

Casteele et al. 2001). Exact tests as described by Guo and Thompson (1992) as implemented in the program GENEPOP (Raymond and Rousset 1995) were used to ensure that loci were independent and that observed genotype frequencies accurately reflected expectations under Hardy-Weinberg equilibrium. Subsequent analyses were carried out for TB-positive and non-infected animals for each of the three years. Allele frequencies, allelic diversity, and heterozygosity were calculated using GENEPOP (Raymond and Rousset 1995).

Molecular marker data were used to estimate the degree of relatedness among individuals to statistically infer genealogical relationships among animals. The pairwise coefficient of relatedness is the probability that 2 alleles at a locus from 2 different individuals are identical by descent. For diploid systems, the coefficient of relatedness is expected to be equal to 2 times the coefficient of coancestry, and is estimated by the fraction of alleles that 2 individuals share that are identical by descent. Marker-based relatedness estimates are typically characterized by large errors of inference (Ritland 1996, Lynch and Ritland 1999). The performance of different estimators varies with the shape of the allele frequency distributions, the number of alleles per locus, and the characteristics of the population (i.e. age, sex composition) under study (Van de Casteele et al. 2001). Because of the differential performance of relatedness estimators, relatedness was calculated using 3 different estimators: the regression-based estimator developed by Queller and Goodnight (1989) implemented in the program KINSHIP, the allele sharing method described by Bowcock et al. (1994), and the regression-based method-of-moments estimator developed by Lynch and Ritland (1999) implemented in the program DELRIOUS (Stone and Bjorklund 2001).

KINSHIP 1.3 was used to calculate estimates of relatedness (QGr_{xy} values) between all pairs TB-positive deer and estimates of relatedness between all pairs of non-infected deer for 1998, 1999, and 2000. The coefficient of relatedness (QGr_{xy}) derived by Queller and Goodnight (1989) is represented in the following equation:

$$QGr_{xy} = \frac{\sum_k \sum_a \left(p_y - p^*\right)}{\sum_k \sum_a \left(p_x - p^*\right)}$$

For each pairwise estimate, the coefficient of relationship is summed over all loci, k and all alleles at each locus, a. The frequency of an allele in individual x (0.5 or 1) is represented by p_x while p_y is the frequency of that same allele in individual y (0, 0.5, or 1). The mean frequency of a given allele in the entire population is represented by p^*. The statistical bias present in p^* is corrected for by omitting the 2 individuals being compared. The resulting r_{xy} calculation is an estimate of the proportion of alleles 2 individuals share that are identical by descent (IBD). Estimates of relatedness between 2 individuals range from 1 (all alleles IBD) to –1. In a randomly mating population, mean relatedness is expected to be zero. Negative estimates of relatedness indicate that 2 individuals share fewer alleles IBD than expected based on the population mean. Standard errors associated with estimates of pairwise r_{xy} values were estimated through jackknifing across loci using RELATEDNESS 4.2c (Queller and Goodnight 1989).

Pairwise relatedness estimates between all TB-positive deer and between all non-infected deer were also calculated using the similarity measure described by Bowcock et al. (1994):

$$P_S = \frac{\sum P_s}{\frac{L}{2L}}$$

The similarity between the multi-locus genotype of 2 individuals, P_S, is evaluated based on the sum of the number of alleles 2 individuals share, P_s, across all loci, L, divided by 2 times the number of loci screened. Similarity values range from 0 (no alleles shared) to 1 (all alleles shared).

Relatedness estimates were also calculated using Lynch and Ritland's (1999) statistic as implemented in the program DELRIOUS (Stone and Bjorklund 2001). The Lynch and Ritland (1999) estimator is:

$$LR\, r_{xy} = \frac{p_a(S_{bc} + S_{bd}) + p_b(S_{ac} + S_{ad}) - 4p_a p_b}{(1 + S_{ab})(p_a + p_b) - 4p_a p_b}$$

For each pairwise estimate of relatedness, individual x possesses alleles a and b and the second individual, y, possesses alleles c and d. If individual x is homozygous, $S_{ab} = 1$ and $S_{ab} = 0$ if it is heterozygous. If allele a from individual x is the same as allele c from individual y then, $S_{ac} = 1$, while $S_{ac} = 0$ if alleles a and c are different. There are 6 different S values corresponding to the 6 possible combinations of the 4 alleles. The frequencies of alleles a and b in the population are represented by p_a and p_b respectively. The estimators are calculated on the basis of conditional probabilities involving allele frequencies and similarity measures. For each calculation, one individual serves as the reference and the probability of its locus-specific genotype in the second individual is

conditioned on the reference individual (Lynch and Ritland 1999). For multiple loci, the estimators are multiplied by locus-specific weights to account for sampling variance differences among loci (Stone and Bjorklund 2001). Lynch and Ritland's LRr_{xy} values range from -1 (completely unrelated) to 1 (genetically identical).

Relatedness Among TB-positive and Non-infected Deer Across All of DMU452

TB-positive deer harvested in DMU452 in 1998, 1999, and 2000 whose harvest location was identified to 2.6-km^2 were selected (Table 3). In order to control for geographic separation among individuals, a non-infected deer was randomly selected from the same or adjacent 2.6-km^2 area from which each TB-positive deer was harvested. Relatedness between all pairs of TB-positive deer and between all pairs of non-infected deer was calculated using the 3 estimators described above.

Pairwise similarity data are interdependent and analysis using standard parametric or nonparametric tests is not appropriate (Dietz 1983). Permutation tests were used to evaluate significance of differences between relatedness estimates for TB-positive and non-infected deer for each year (Wayne et al. 1991, Lehman et al. 1992). A SAS-based program (A. Saxton, University of Tennessee, unpublished software) was used to first compute the difference between the means of the 2 groups. The data from the 2 groups were pooled, and these data were randomly subsampled 500 times. For each subsampling, the difference between the observed and theoretical mean calculated from the pooled data was calculated and the one-tailed probability of the two means being different was tested (Ratnayeke et al. 2002). Based upon the hypothesis that the genealogical model played an important role in TB transmission among deer, it was

predicted that mean pairwise estimates of relatedness would be greater for TB-positive deer than for non-infected deer.

The relationship between genetic differentiation and geographic distance was calculated for all pairs of TB-positive deer and non-infected deer and compared in order to more explicitly examine the role of genealogical relationships in disease transmission. Cavalli-Sforza Edwards chord distances (1967) were calculated for all pairs of TB-positive deer and all pairs of non-infected individuals for each year using BIOSYS (Swoford and Selander 1981). X,Y (UTM) coordinates were used to calculate Euclidean geographic distances between individuals using the program PASSAGE (Rosenberg 2000). Mantel tests (Mantel 1967) of correlations between matrices of genetic and geographic distance between pairs of TB-positive deer and pairs of non-infected deer for each year were performed using the program PASSAGE (Rosenberg 2000). Significant relationships between genetic and geographic distance were evaluated through randomization tests in which the order of elements within one matrix was randomly permutated 999 times (Smouse et al. 1986). If there is a strong (non-random) association between the 2 matrices, the iterations will be more extreme than the observed association. A significant relationship between the degree to which TB-positive deer were genetically differentiated and their geographic distance from one another relative to that among non-infected deer would indicate that the probability of infection with TB is not random, but rather a function of genealogical relationships among infected individuals.

Relatedness Among TB-positive and Non-infected Deer at Distances Consistent With Deer Movement in Northeast Michigan

Mean relatedness between TB-positive deer was compared to mean relatedness between non-infected deer at distances consistent with deer movement distances in DMU452 using the Bowcock et al. (1994) and Queller and Goodnight (1989) estimators described above for each year. Mean relatedness between pairs of TB-positive deer separated by several geographic distances was calculated. There were many more non-infected deer than TB-positive deer (Table 3). Mean relatedness among non-infected deer was calculated by randomly selecting a number of non-infected deer equivalent to the number of TB-positive deer at each distance class. This process was repeated 5,000 times. The probability that TB-positive deer were more closely related than non-infected deer at a given distance class was calculated as the number of times the mean relatedness among TB-positive deer was greater than the mean relatedness among non-infected deer divided by 5,000 (Manly 1997). We expected that if the probability of infection with TB was not random, but rather a function of genealogical relationships, that for deer separated by distances in which all deer could conceivably come into contact, mean relatedness among TB-positive deer would be higher than relatedness among non-infected deer.

Results

Hardy Weinberg tests after Bonferroni correction indicated an excess of homozygotes at one of 11 loci ($p < 0.005$). One pair of 55 pairwise comparisons of loci were in linkage disequilibrium ($p < 0.0009$). Both excess homozygosity, often indicative

of null alleles, and gametic disequilibrium reduce the ability to resolve relationships among individuals. The power to accurately resolve pedigree relationships among individuals, however, increases with the number of loci used. Therefore, data from all loci were used for estimation of relatedness between individuals.

Heterozygosity and allelic diversity were high for both TB-positive and non-infected groups of deer for all years, both prior to and following the ban on artificial feeding (Table 4). Allelic diversity at each locus was high ranging from 9-22 alleles per locus (mean=15.4± 1.1). Loci that possess large numbers of alleles are useful in determining genealogical relationships among individuals in close proximity (Parker et al. 1998). When individuals share an allele at a highly polymorphic locus there is an increased probability that the alleles are identical by descent.

Relatedness Among TB-positive and Non-infected Deer Across All of DMU452

Mean relatedness was significantly higher among TB-positive deer than among non-infected deer over the whole of DMU452 both before (1998) and after management changes (2000) as measured by both the Queller and Goodnight (1989) and Bowcock et al. (1994) estimators (Table 5). The distribution of relatedness values between pairs of TB-positive deer was skewed towards higher relatedness than was the distribution between pairs of non-infected deer both prior to and following management changes as measured by Queller and Goodnight's (1989) coefficient of relatedness (QGr_{xy}) and Bowcock et al.'s (1994) similarity index (Table 5) (Figure 3, 4). No significant differences were detected for any year using Lynch and Ritland's (1999) coefficient (Table 5) (Figure 5).

Table 4. Mean heterozygosity and allelic diversity (±SE) among TB-positive deer (+) and among non-infected deer (-) for 1998-2000.

Year	TB Status	Heterozygosity	Allelic Diversity
1998	+	0.788 (0.026)	12.2 (1.0)
1998	-	0.835 (0.024)	12.5 (1.1)
1999	+	0.805 (0.031)	11.6 (0.9)
1999	-	0.837 (0.028)	11.9 (0.9)
2000	+	0.843 (0.032)	11.0 (0.8)
2000	-	0.821 (0.023)	11.5 (0.8)

Table 5. Mean pairwise estimates of relatedness among TB-positive deer (+) and among non-infected deer (-) during 1998-2000 as measured by Queller and Goodnight's (1989) QGr_{xy} (±SE), Bowcock et al.'s (1994) Similarity, and Lynch and Ritland's (1999) LRr_{xy}. Associated p-values are based on permutation tests of differences in mean relatedness between TB-positive deer and non-infected deer for each measure for each year.

Year	QGr_{xy}	Similarity	LRr_{xy}
1998 +	0.025 (0.010)	0.238	-0.020
1998 -	-0.015 (0.009)	0.229	-0.016
p-value	<0.001	<0.001	0.744
1999 +	0.002 (0.015)	0.236	-0.025
1999 -	0.001 (0.017)	0.248	-0.023
p-value	0.446	1	0.652
2000 +	0.014 (0.025)	0.255	-0.034
2000 -	-0.009 (0.018)	0.236	-0.033
p-value	<0.001	<0.001	0.544

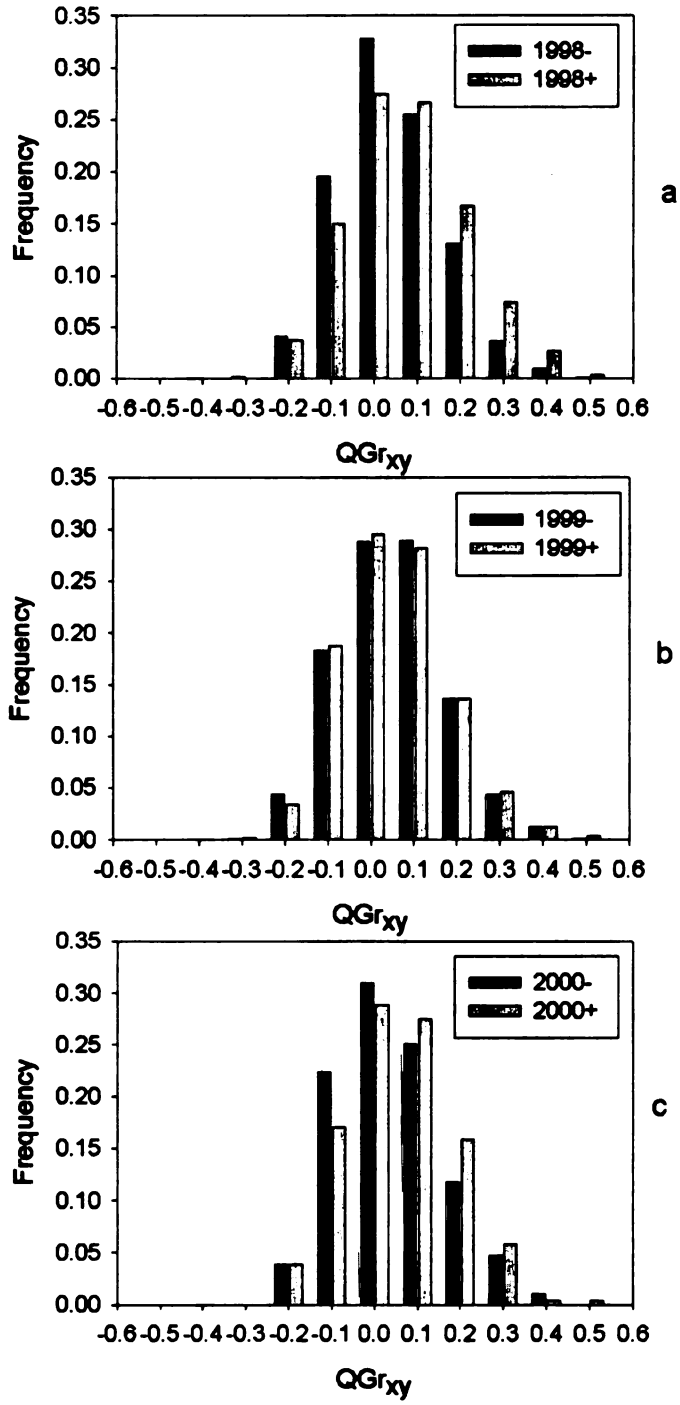

Figure 3. Distribution of Queller and Goodnight's estimates of relatedness (QGr_{xy}) among TB-positive deer and among non-infected deer in the year prior to the ban on artificial feeding (a), in the year in which artificial feeding was banned (b), and in the year following the ban on artificial feeding (c)

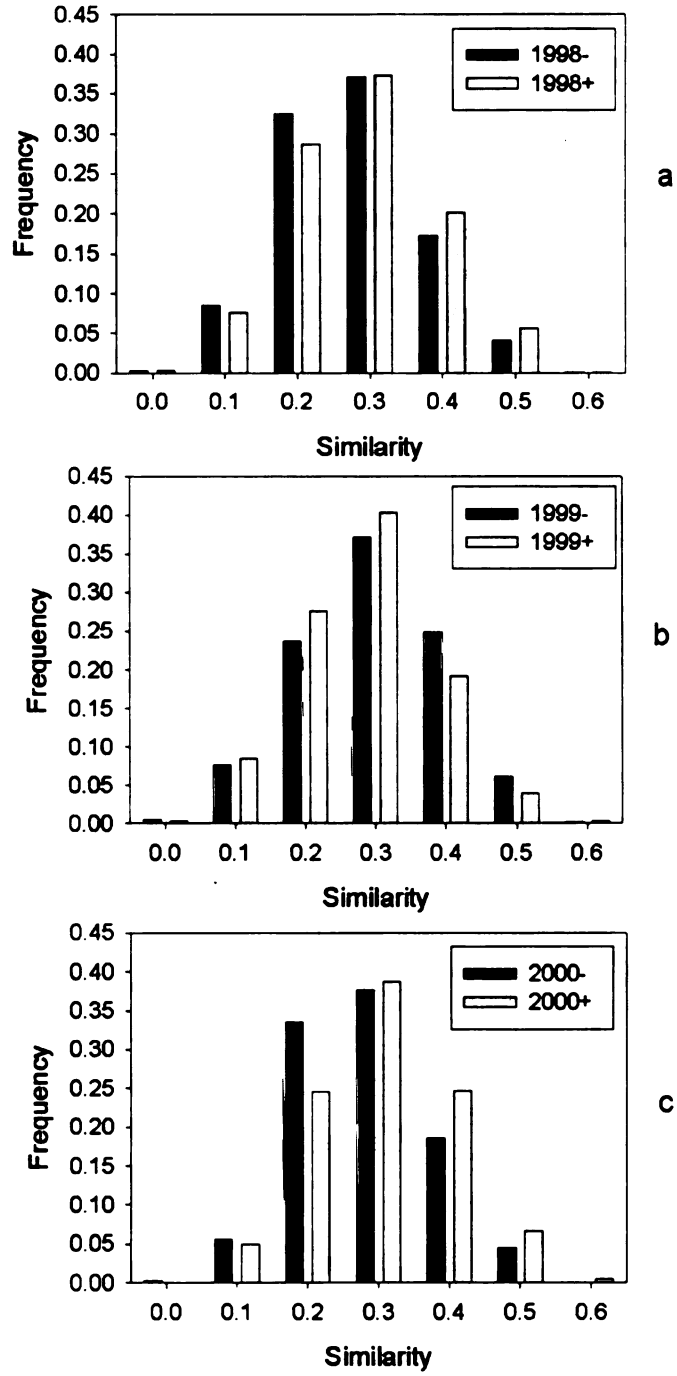

Figure 4. Distribution of Bowcock et al.'s Similarity values among TB-positive deer and among non-infected deer in the year prior to the ban on artificial feeding (a), in the year in which artificial feeding was banned (b), and in the year following the ban on artificial feeding (c).

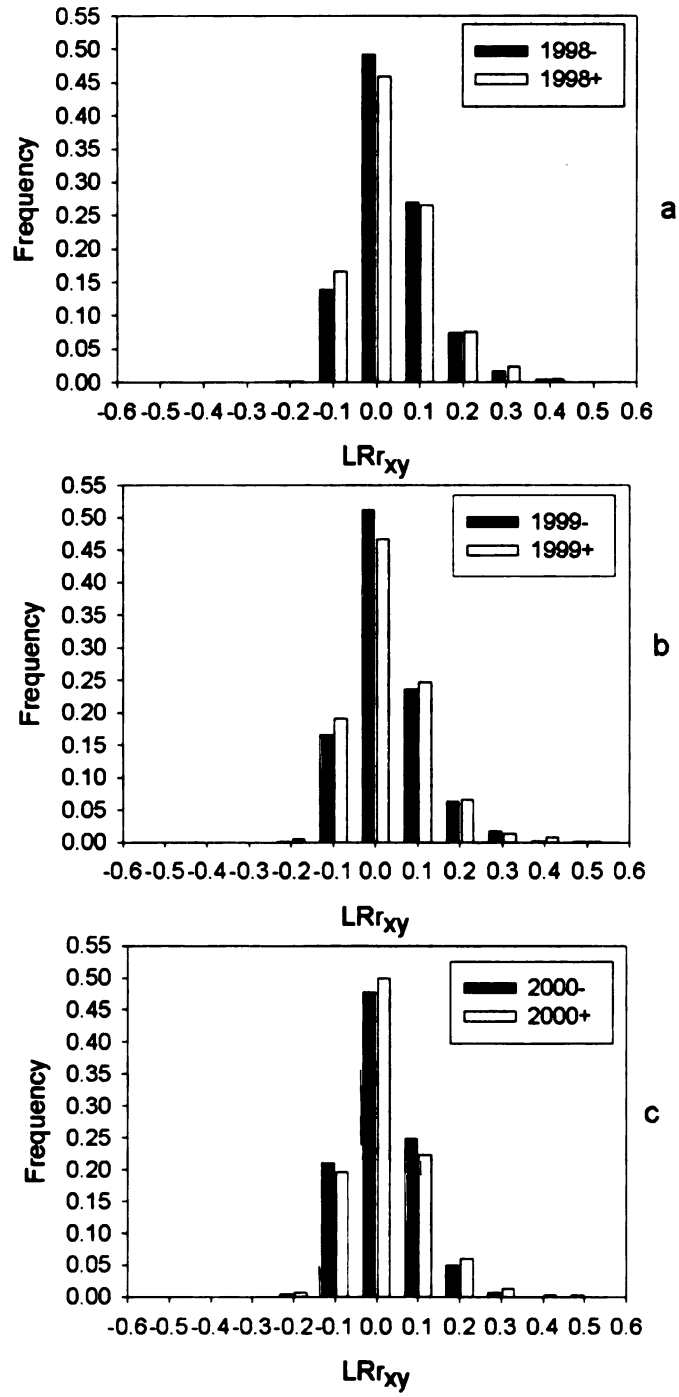

Figure 5. Distribution of Lynch and Ritland's estimates of relatedness (LRr_{xy}) among TB-positive deer and among non-infected deer in the year prior to the ban on artificial feeding (a), in the year in which artificial feeding was banned (b), and in the year following the ban on artificial feeding (c).

There was a significant correlation between the degree to which TB-positive deer were genetically differentiated and their geographic distance from one another following the ban on artificial feeding (2000) and a marginal relationship when artificial feeding was occurring (1998) (Table 6). TB-infected deer that were close to one another in space were more genetically similar than were TB-positive deer separated by larger geographic distances. There was not a significant relationship between genetic differentiation and geographic distance for non-infected deer before or after management actions (Table 6). Non-infected deer that were close to one another in space were no more similar genetically than non-infected deer separated by larger distances. The lack of genetic similarity between non-infected deer that were geographically proximal relative to genetic similarity between non-infected deer separated by larger geographic distances indicates that the non-infected deer were random samples of the deer population. The increasing degree of genetic differentiation between TB-positive deer with increasing geographic distance between them, however, indicates that TB-positive deer were not a random sample of the entire deer population. Specifically, TB-infected deer that were geographically close to one another in space were highly genetically similar to one another relative to TB-infected deer further apart in space indicating that the risk of infection with TB is not random, but rather is a function of genealogical relationships.

Relatedness Among TB-positive and Non-infected Deer at Distances Consistent With Deer Movement in Northeast Michigan

Mean relatedness among TB-positive deer was higher than mean relatedness among non-infected deer harvested in the same 2.6-km^2 both prior to (1998) and

Table 6. Results of Mantel tests of correlations between genetic and geographic distance including results of permutation tests of significance and associated p-values for TB-positive deer (+) and for non-infected deer (-) during 1998-2000.

		# of Iterations out of 999		
Year	Correlation	< Observed	> Observed	p-value
1998 +	0.079	937	62	0.063
1998 -	-0.054	177	822	0.823
1999 +	0.009	550	449	0.450
1999 -	-0.030	290	709	0.710
2000 +	0.144	957	42	0.043
2000 -	-0.061	216	783	0.784

coincident with the ban on artificial feeding and increased hunting (1999, 2000) for both the Queller and Goodnight (1989) and Bowcock et al.'s (1994) measures (Figure 6, 7) (Table 7, 8). In all years, there was a general pattern of higher relatedness at shorter distances and decreasing relatedness with increasing separation distance (Figure 6, 7). In addition in 2000, mean relatedness among TB-positive deer was consistently higher than mean relatedness among non-infected deer up to distances consistent with the average maximum distance of deer movement (<20km) in northeast MI for both Queller and Goodnight's (1989) QGr_{xy} and Bowcock et al.'s (1994) similarity (Figure 6, 7) (Table 7, 8). At distances beyond which deer generally move in DMU452, relatedness between TB-positive and between non-infected deer declined and did not differ (Figure 6, 7). The higher relatedness among TB-positive deer relative to relatedness among non-infected deer separated by distances in which all individuals could conceivably come into contact indicates that all deer were not equally likely to become infected with TB.

Discussion

Estimates of genetic relatedness among TB-positive deer were higher than were comparable estimates among non-infected deer both when artificial feeding was occurring (1998) and after it had been banned (2000) as measured by 2 of 3 estimators. Results were consistent both independent of the geographic proximity of individuals and at distances consistent with what is known about deer movement in northeast MI. The greater genetic similarity among TB-positive deer relative to non-infected deer indicates that the probability of TB infection is non-random, and that genealogical relationships play an important role in the maintenance of TB. The role that genealogical relationships

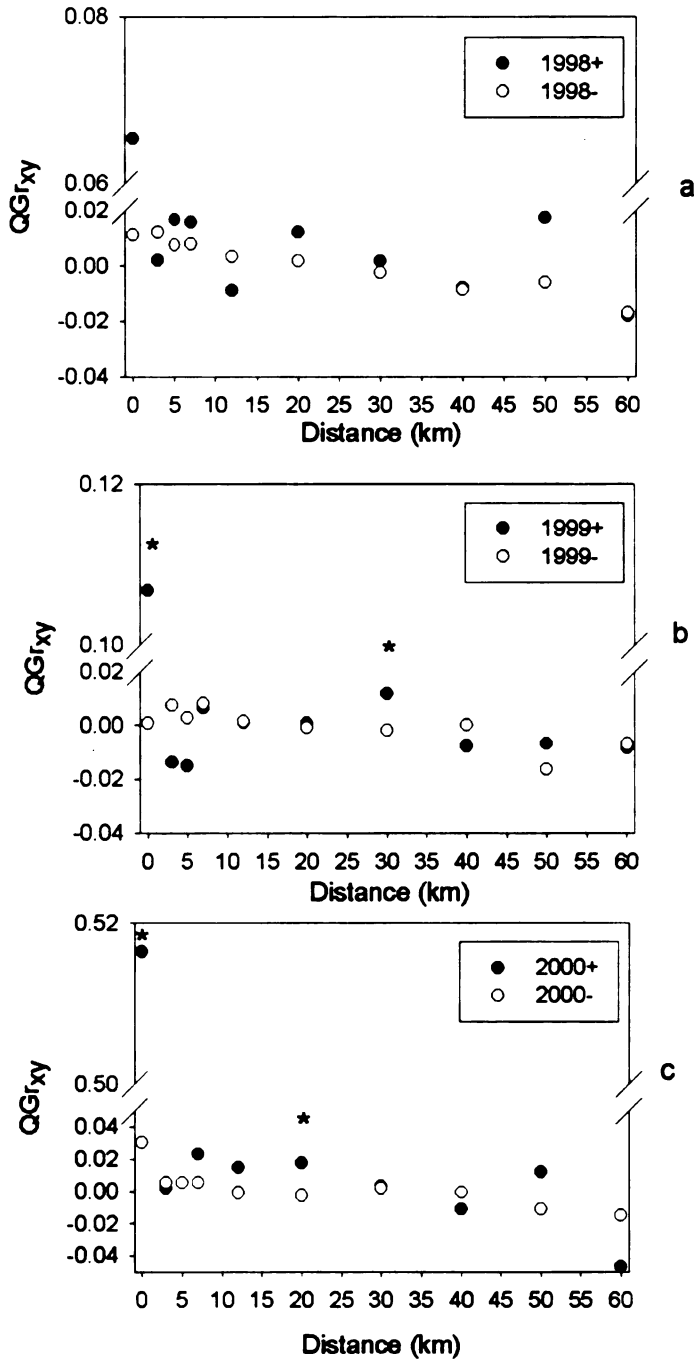

Figure 6. Queller and Goodnight estimates of relatedness (QGr_{xy}) among TB-positive deer and among non-infected deer at different distances of geographic separation in the year prior to the ban on artificial feeding (a), in the year in which artificial feeding was banned (b), and in the year following the ban on artificial feeding (c). Asterisks indicate significant differences in mean pairwise relatedness between TB-positive and non-infected deer ($p<0.05$).

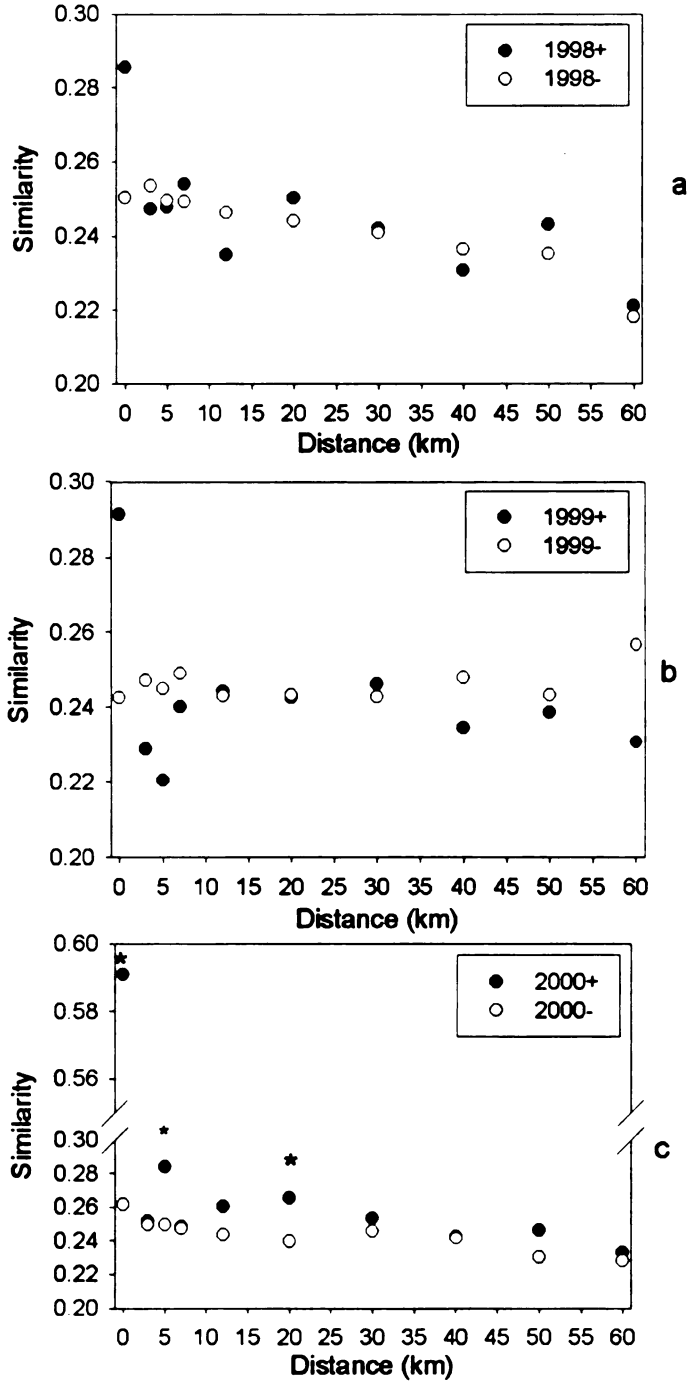

Figure 7. Bowcock et al. Similarity values among TB-positive deer and among non-infected deer at different distances of geographic separation in the year prior to the ban on artificial feeding (a), in the year in which artificial feeding was banned (b), and in the year following the ban on artificial feeding (c). Asterisks indicate significant differences in mean pairwise relatedness between TB-positive and non-infected deer ($p<0.05$).

Table 7. Mean QGrxy and associated p-values between TB-positive deer (+) and between TB-negative deer (-) at different distances of geographic separation (km).

Distance	1998 +	1998 -	p-value	1999 +	1999 -	p-value	2000 +	2000 -	p-value
0	0.065	0.011	0.1	0.107	0.001	0.03	0.516	0.03	0.01
3	0.002	0.012	0.69	-0.014	0.008	0.72	0.002	0.005	0.5
5	0.017	0.007	0.32	-0.015	0.003	0.77	0.05	0.005	0.1
7	0.016	0.008	0.31	0.006	0.008	0.54	0.023	0.005	0.28
12	-0.009	0.003	0.91	0.001	0.001	0.52	0.015	-0.001	0.14
20	0.012	0.002	0.04	0.001	-0.001	0.42	0.018	-0.003	0.03
30	0.002	-0.002	0.22	0.012	-0.002	0.03	0.003	0.002	0.46
40	-0.008	-0.009	0.45	-0.008	0	0.75	-0.011	-0.001	0.79
50	0.017	-0.006	0.02	-0.007	-0.016	0.29	0.012	-0.011	0.13
60	-0.018	-0.017	0.52	-0.008	-0.007	0.55	-0.047	-0.015	0.72

Table 8. Mean similarity and associated p-values between TB-positive deer (+) and between non-infected deer (-) at different distances of geographic separation (km).

Distance	1998 +	1998 -	p-value	1999 +	1999 -	p-value	2000 +	2000 -	p-value
0	0.286	0.25	0.11	0.291	0.243	0.08	0.591	0.261	0.01
3	0.247	0.253	0.66	0.229	0.247	0.77	0.251	0.249	0.48
5	0.248	0.249	0.54	0.22	0.245	0.93	0.284	0.25	0.01
7	0.254	0.249	0.32	0.24	0.249	0.78	0.249	0.247	0.48
12	0.235	0.246	0.96	0.244	0.243	0.43	0.26	0.244	0.06
20	0.25	0.244	0.06	0.243	0.243	0.54	0.266	0.24	0.01
30	0.242	0.241	0.4	0.246	0.243	0.28	0.253	0.245	0.13
40	0.231	0.236	0.9	0.234	0.248	0.97	0.242	0.242	0.48
50	0.243	0.235	0.14	0.239	0.243	0.63	0.246	0.231	0.13
60	0.221	0.218	0.35	0.231	0.257	0.94	0.233	0.228	0.43

play in the risk of infection with TB is increasingly important following the ban on artificial feeding that dramatically reduced or eliminated the risk of infection resulting from contacts at artificial feeding sites. Regardless of management actions aimed at decreasing population density, crowding, and contacts among deer at food piles, data imply that matrilineal groups likely serve as reservoirs of TB infection within the deer population. The ban on artificial feeding and reduction in total population density reduce contacts among individuals and thus disease transmission across matrilines at feeding sites, but these actions alone are unlikely to eradicate TB. Genetic data suggest that management must also focus on aspects of social and kin structure facilitating infection.

Unlike results found using the Queller and Goodnight (1989) and Bowcock et al. (1994) measures, analyses using the Lynch and Ritland (1999) measure did not indicate significant differences in relatedness between TB-positive and non-infected deer (Table 5) (Figure 5). The performance of different estimators varies with the shape of the allele frequency distributions, the number of alleles per locus, and the population composition (Van de Casteele et al. 2001). Van de Casteele et al. (2001) found that from known pedigrees, Queller and Goodnight's (1989) coefficient of relatedness more accurately estimated relationships than did Lynch and Ritland's (1999) estimator for highly polymorphic markers. Lynch and Ritland's estimator also was not as accurate as the Queller and Goodnight estimator for multiple locus analyses when the number of alleles varies across loci (Van de Casteele et al. 2001). The 11 loci used in these analyses were extremely polymorphic ranging from 9-22 alleles per locus (mean=15.4± 1.1) (Table 4). Due to the characteristics of the loci used for these analyses, the Lynch and Ritland

(1999) coefficient of relatedness was not likely the best estimator of relatedness for these data.

Data from 1999 did not indicate a higher degree of relatedness among TB-positive deer than among non-infected deer (Table 5) (Figure 3, 4). TB-positive deer harvested in the same 2.6-km^2, however, were more highly related than were non-infected deer in 1999 (Table 7, 8) (Figure 6, 7). In addition to the ban on artificial feeding, antlerless hunting quotas were increased in 1999. Increased harvest of females could have resulted in a larger number of females or younger animals used in the 1999 analysis than in 1998 or 2000. A review of the samples used in the 1999 analysis, however, did not indicate a bias towards females (36 females, 41 males). A one-way analysis of variance indicated that the age of sampled deer did not differ as a function of year ($F= 0.76$, $df= 2$, $p= 0.47$). The only difference in the 1999 samples, unlike samples from 1998 and 2000, was that non-infected deer were significantly younger than TB-positive deer ($t= -3.43$, $df= 75$, $p= 0.001$). Given that TB-positive deer are consistently older than non-infected deer (O'Brien et al. 2002), the younger age of non-infected deer samples in 1999 does not explain why results do not agree with those from the years prior to and following 1999.

Recently, numerous studies have focused on determining how host ecology influences the dynamics of disease transmission in natural populations (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). There are often differences in the frequency of infection among demographic segments of wildlife populations (Mills and Childs 1998). Patterns of cowpox infection in rodents vary as a function of age and sex (Hazel et al. 2000). The difference between male and female tick dispersal and host specificity may be important

in transmission of Lyme disease (Meeus et al. 2002). Differences among age groups and sex in infection with Puumala virus were used to infer how behavior influenced disease transmission among bank voles (Escutenaire et al. 2002). Boone et al. (1998) found that adult male deer mice had a higher prevalence of hantavirus than did other segments of the population inferring that wounding during territorial disputes was a major cause of disease transmission (Boone et al. 1998). In addition, they found a complex non-linear relationship between density and disease prevalence (Boone et al. 1998). Above a certain density, there was no predictive correlation between density and hantavirus prevalence (Boone et al. 1998).

Complex social behavior can also influence disease transmission in wildlife. The rate of bovine TB transmission in brushtail possums increased markedly when highly social possums were infected compared to transmission rates among randomly mixed possums (Corner et al. 2002). Transmission risk depended on the spatial proximity of individuals and rates of social interaction (Corner et al. 2002). The seasonality in rabies incidence in jackal populations can be explained by the seasonality of their social behavior (Loveridge and Macdonald 2001). Increased contact among neighboring groups of jackals during mating and dispersal was correlated with increased incidence of rabies while decreased home ranges and neighbor overlap following breeding were correlated with decreased rabies prevalence (Loveridge and Macdonald 2001). Changes in the population density of badgers in Great Britain, the main reservoir there for bovine tuberculosis, were related to incidence of TB in cattle (Hutchings and White 2000). Hutchings and White (2000) found that human culling of badger populations decreased population densities sufficiently to disrupt their territorial behavior and communal latrine

use. Rather than eliminating disease transmission, culling resulted in a wider spatial dissemination of badger urination and an increased probability of cattle contact with urine-contaminated grass (Hutchings and White 2000). Clearly, knowledge of host ecology can increase understanding of mechanisms of disease transmission in wildlife.

It has been demonstrated based upon estimates of genealogical relationships that the probability of infection with TB is not random. Rather, matrilineal groups likely serve as reservoirs of TB infection that is facilitated by deer's strong social structure. The close association and high rate of contact that occurs among individuals within matrilines seems to be the most likely mechanism by which genealogical relationships influence disease transmission. There is also the possibility, however, that the higher relatedness among TB-infected individuals relative to relatedness among non-infected individuals is due to genetic susceptibility to TB infection. Additionally, the fact that groups of related individuals share a common environment which in turn might pose a risk of TB infection cannot be discounted.

This is the first study of its kind to use molecular markers and genealogical relationships to infer wildlife ecology's role in transmission dynamics between host and pathogen populations. Data clearly demonstrate the utility of using molecular markers and population genetic theory to aid managers in the development of timely and effective strategies for the control of wildlife disease.

CHAPTER 3

WHITE-TAILED DEER SPATIAL GENETIC STRUCTURE PRIOR TO AND COINCIDENT WITH CHANGES IN ARTIFICIAL FEEDING REGULATIONS

Spatial Structure in White-Tailed Deer

Humans can affect animal social and demographic structures that can affect levels of genetic diversity within and among wildlife populations (Chesser 1983, McCullough and Chesser 1987, VanDenBussche et al. 1987, Kilgo et al. 1998, Trombulak et al. 2000). Disturbances such as habitat loss and fragmentation, hunting, and artificial feeding can change animal spatial dispersion, movements, and foraging behavior (Kilgo et al. 1998, Trombulak et al. 2000). White-tailed deer (*Odocoileus virginianus*) often live in close proximity to humans and are impacted by anthropogenic influences (Scribner et al. 1997).

Deer are large, highly mobile mammals and might be expected to show little spatial structuring at a microgeographic scale due to their potential to disperse long distances, large home ranges, and seasonal migration (McCullough 1979, Marchinton and Hirth 1984). Characteristics of white-tailed deer ecology, including site fidelity, female philopatry, and a polygamous mating system, however, may explain the documentation of spatial genetic structuring in many white-tailed deer populations (Cronin et al. 1991, Scribner 1993, Scribner et al. 1997, Purdue et al. 2000).

White-tailed deer have a matriarchal social structure in which females live in multi-generational female groups (Hawkins and Klimstra 1970, McCullough 1979, Marchinton and Hirth 1984). Hawkins and Klimstra (1970) found that 87% of yearling females remained philopatric and were observed with other group members in greater

than 60% of all observations. In contrast, 80% of yearling males were found to disperse (Hawkins and Klimstra 1970). For those males that did not disperse, home ranges were typically established an average of 3 miles away from their mothers' home range (Hawkins and Klimstra 1970). Adult and young males are occasionally found in groups of 2-5 during the non-breeding season (Hawkins and Klimstra 1970, Marchinton and Hirth 1984, McCullough 1979). However, during the breeding season, which runs from September through December, adult males are solitary and establish breeding hierarchies (Hawkins and Klimstra 1970, Marchinton and Hirth 1984, McCullough 1979). Older, dominant males are presumed to obtain a greater proportion of breeding opportunities than younger males (Hawkins and Klimstra 1970, Marchinton and Hirth 1984, McCullough 1979).

Female philopatry, male-biased dispersal, and non-random mating create genetic substructure within populations (Chesser 1991a). A matrilineal social system in which females are philopatric to natal areas over multiple years creates spatial genetic structure over microgeographic scales (Chesser 1991a, Matthews & Porter 1993). Philopatry leads to high coancestry among individuals within social lineages (θ) relative to coancestry among individuals from different social lineages (α) (Chesser 1991a). Male breeding hierarchies can either reinforce divergence or homogenize genetic diversity in a matrilineally-structured social system (Chesser 1991a,b, Matthews and Porter 1993). A situation in which a single male mates with most or all females in a matriline both increases coancestries within matrilines and the differentiation among matrilines (Chesser 1991a,b). Differentiation among matrilines decreases, however, when all males in a population have relatively equitable mating opportunities, more than one male mates with

females within a matriline, or males mate with females in several matrilines (Chesser 1991a,b). Changes in local white-tailed deer breeding structure due to high rates of population turnover, young age structure, sex-biased gene flow, and method of harvest can alter spatial genetic structure (Scribner et al. 1985, Scribner 1993, Scribner et al. 1997).

White-Tailed Deer in Michigan

In Michigan (MI), before human settlement, white-tailed deer were most abundant in the southern part of the state where the habitat primarily consisted of open oak and hickory forests ideal for deer (Langenau 1994, MDNR 1993). In the northern lower peninsula of MI, forests of primarily pine and hardwood that did not provide optimal habitat resulted in low carrying capacity for deer (MDNR 1993). However, during the early 20th century, numerous hunting clubs were established in northeast MI. Due to high hunting demand and shortage of natural browse, artificial feeding was widely used to enhance deer population numbers. Artificially high deer densities resulting from artificial feeding practices has the potential to have negative effects on the natural vegetation and on the deer population itself in terms of increased transmission and prevalence of disease (Chouinard and Filion 2001, Miller et al. 2003).

The white-tailed deer population in the northeast lower peninsula of MI (referred to here as Deer Management Unit 452 (DMU452)) is infected with bovine tuberculosis (*Mycobacterium bovis*) (TB). Higher prevalence of TB in this region relative to the rest of MI is likely due to the region's long history of artificial feeding (Schmitt et al. 1997).

Artificial food sources have resulted in high deer densities (>35 per mi^2), crowding, and high rates of contact among deer over food (Schmitt et al. 1997). In an effort to decrease TB transmission among deer, artificial feeding was banned in DMU452 in 1999. In addition to facilitating TB transmission, artificial feeding is likely to have affected deer spatial genetic structure by altering deer movement and breeding behavior. Knowledge of deer spatial genetic structure as measured by spatial variation in gene frequencies can be useful in predicting the distance of gene flow and thus the transmission and distribution of TB across DMU452.

In order to characterize the effect of artificial feeding on deer population genetic structure and the potential for disease transmission, the degree of variation in allele frequencies among groups of deer within 2 regions of DMU452 that have different histories of artificial feeding was estimated (Figure 8). It would be impossible to directly observe the movement, behavior, and relationships of the entire white-tailed deer population in DMU452. In the absence of known pedigree relationships, the degree of spatial variation in allele frequencies and gene flow can be estimated indirectly by assaying geographic variation in the frequency of heritable genetic markers (Scribner et al. 1997).

Spatial autocorrelation techniques are often used to describe non-random patterns in spatial distributions of alleles or genotypes (Sokal and Oden 1978, Cliff and Ord 1981, Sokal and Jacquez 1991, Epperson 1993, Sokal et al. 1997, Koenig 1999). Specifically, spatial autocorrelation is a measure of the degree of interdependence between variables (Cliff and Ord 1981). Autocorrelation analysis has advantages in describing population structure over standard techniques such as Fst, a descriptor of the overall degree of

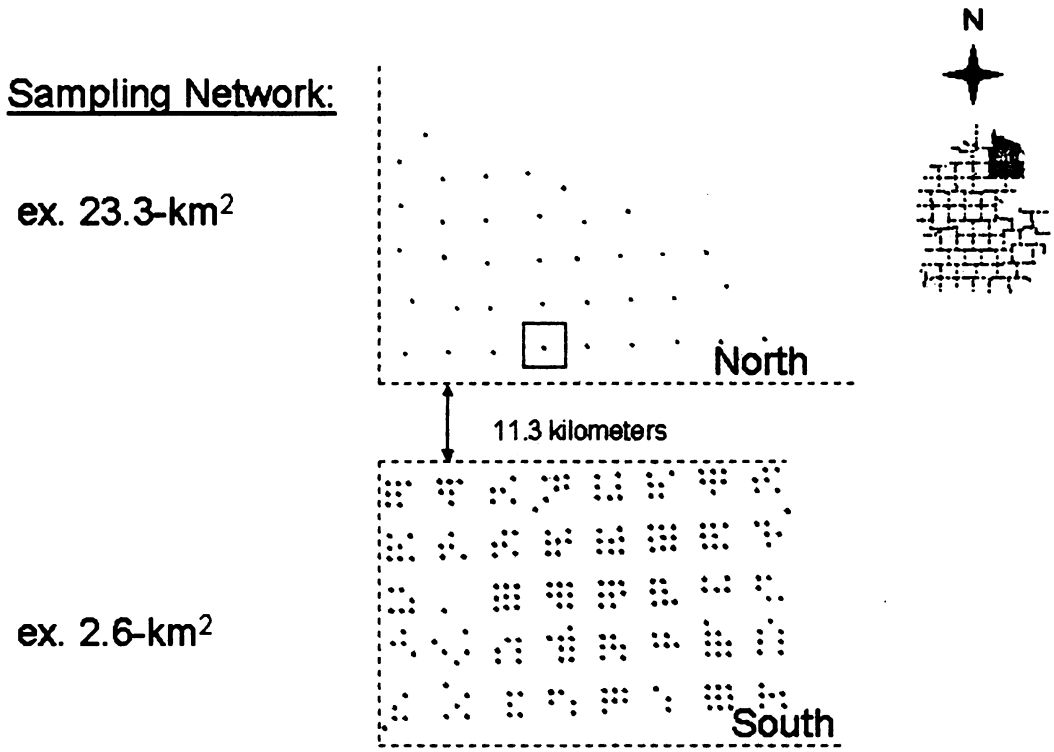

Figure 8. Map of northeast MI (DMU452) and networks for the analysis of spatial genetic structure among "subpopulations" of deer. In the North region is a depiction of the network of 23.3-km^2 quadrats. Each point indicates the center of the 23.3-km^2 quadrat and the box is an example of a single 23.3-km^2 quadrat. In the South region is an example of the network of 2.6-km^2 quadrats.

variance among locations, because it explicitly describes patterns of genetic variation over space (Barbujani 1987, Sork et al. 1999). Spatial autocorrelation techniques can be used to characterize the geographic variation in biological populations and to make inferences concerning ecological or demographic features of populations and the landscape they inhabit that lead to non-random spatial associations (Oden and Sokal 1986, Sokal et al. 1997).

Random mixing of large numbers of deer at feeding sites contrasts with a natural free-ranging population in which deer, especially females, interact primarily with their relatives (Hawkins and Klimstra 1970). Artificial feeding disrupts the spatial segregation of matrilines by bringing groups of deer into the same spatial location. Specifically, in the absence of artificial feeding, individuals from several matrilines may coexist in a common area, but the degree of social interaction and probability of contact among these individuals increases when the population is artificially fed. It was hypothesized that the greater the intensity of artificial feeding, the greater homogenization of population genetic diversity. Due to intensive artificial feeding, deer in southern areas of DMU452 (South) were expected to exhibit weaker spatial genetic structuring relative to the less heavily artificially fed northern DMU452 deer (North). Following the ban on artificial feeding, it was hypothesized that there would be a stronger relationship between genetic differentiation among groups of deer and their geographic distance from one another in both the North and South regions (2000).

Methods

DMU452 was divided into 2 regions on the basis of differing histories of artificial feeding intensity (Figure 8). One region referred to as the South region was located in the region of most intensive artificial feeding (in terms of the number and size of feeding sites) and the highest incidence of TB (319 TB-positive deer identified from 1995-2000). The South region was located primarily in Alpena and Montmorency counties and extended south into the north-most sections of Alcona and Oscoda counties and covered 2,323-km^2 (Figure 8). Deer throughout South have been intensively artificially fed (hundreds of thousands of kilograms per year) for several decades (Hickling 2002, Miller et al. 2003). The second region, referred to as the North region was located in the northern-most part of DMU452 where incidence of TB was lower (5 TB-positive deer identified from 1995-2000). Artificial feeding in this region has historically been lower than in the South region. The North region was located 11.3 kilometers to the north of the South region in southern Presque Isle county and the very northern-most portions of Alpena and Montmorency counties and covered 1,994-km^2 (Figure 8).

The North and South regions were divided into two different sized networks of "subpopulations" (quadrats) (Figure 8). The first network consisted of 37 (North region) and 40 (South region) approximately 23.3-km^2 quadrats each separated by a 3.2 km buffer (Figure 8). The second network consisted of 2.6-km^2 quadrats (Figure 8). The number of 2.6-km^2 quadrats in each region ranged from 142-258 based on sample availability in each year of the analysis (Table 9). Spatial locations of each quadrat for the 23.3-km^2 network were defined by the X,Y coordinate (UTM) of the centermost 2.6-km^2

Table 9. Number of deer genotyped and number of quadrats in each network for the North and South regions during 1998, 1999, and 2000.

Region	Year	# Individuals	# 23.3-km^2 quadrats	# 2.6-km^2 quadrats
North	1998	644	37	205
North	1999	616	37	176
North	2000	351	37	142
South	1998	654	40	252
South	1999	663	40	258
South	2000	726	40	255

section of each 23.3-km^2 quadrat. The spatial location for quadrats within the 2.6-km^2 network was defined by the UTM location of the center of each 2.6-km^2 quadrat.

Samples of hunter-harvested deer were collected as described in Chapter 1. All deer samples from 1998, 1999, and 2000 (~3660) harvested within the quadrats described above were selected for the following analyses. Because sampling intensity across the network was a function of hunter harvests, sample sizes were not uniform across quadrats of each network (Table 9).

DNA was extracted from deer samples using the QIAGEN DNEasy protocol. Analyses were based on the spatial dispersion of alleles within and among quadrats of deer for both regions at 3 moderately polymorphic loci (9-12 alleles per locus) (IGF-1, OBCAM, and RT-9, Appendices I, II) (Kirkpatrick 1992, Fries et al. 1993, Wilson et al. 1997). Exact tests described by Guo and Thompson (1992) as implemented in the program GENEPOP (Raymond and Rousset 1995) were used to estimate levels of gametic disequilibrium to ensure that loci were independent and to determine whether observed allele frequencies accurately reflected expectations under Hardy-Weinberg equilibrium.

The program GENEPOP was used to estimate allele frequencies, observed and expected heterozygosity, and allelic diversity for each locus for each region for each year (Raymond and Rousset 1995). Allelic richness was calculated using the program CONTRIB in order to adjust allelic diversity measures for sample size differences among years and between regions (Petit et al. 1998). Due to limitations in the capacity of some software, some data in the network of 2.6-km^2 quadrats were grouped in order to fit the dimensions of the software. Four individuals possessing unique or extremely rare alleles

(frequency <0.0001) were removed from subsequent analyses. Additionally, 4 rare alleles (frequency <0.002) at the IGF-1 locus were binned with the next largest size allele and the allele was renamed. The degree of population genetic divergence within each region for each year was assayed by measuring the variation in allele frequencies among all quadrats of deer (Fst) (Weir and Cockerham 1984), and was estimated for each network using the program GENEPOP (Raymond and Rousset 1995).

Genotypes of deer within each quadrat were used to calculate allele frequencies for each quadrat of deer using GENEPOP (Raymond and Rousset 1995). Allele frequencies of deer within quadrats were used to estimate Cavalli-Sforza Edwards chord distances (1967) between quadrats of deer for each network for each region and for each year using the program BIOSYS (Swofford and Selander 1981) or the program PHYLIP 3.5 (Felsenstein 1993). UTM coordinates were used to calculate Euclidean geographic distances between quadrats using the program PASSAGE (Rosenberg 2000). Mantel tests were implemented in the program PASSAGE to estimate the relationship between genetic distance and geographic distance matrices constructed from all pairwise comparisons of quadrats within each region for each year (Rosenberg 2000). A Mantel test examines the relationship between matrices whose values are measures between 2 points or sampling units (Mantel 1967, Fortin and Gurevitch 2001). Significance of correlations between genetic and geographic distance were evaluated through randomization tests in which the elements of one matrix were randomly permuted 1000 times (Smouse et al. 1986). If there is a strong (non-random) association between the 2 matrices, the iterations will be more extreme (biased towards either larger or smaller values) than the observed association.

In addition to characterizing the magnitude of spatial autocorrelation over an entire region, spatial relationships among locations can be characterized at a series of successively larger distance classes (Rosenberg 2000). Mantel tests (Mantel 1967) were used to estimate the degree of genetic differentiation among quadrats of deer separated by several geographic distances for each region for each year using the program PASSAGE (Rosenberg 2000). The normalized Mantel correlation was estimated between a matrix of genetic distances between pairs of quadrats of deer and a weight matrix in which only pairs of quadrats of deer separated by geographic distances within the focal distance class were included. Correlogram plots of the correlation coefficient at each distance class were constructed for each region for each year to summarize patterns of genetic variation in space (Sokal and Oden 1978), and to identify geographic distances at which autocorrelation is high or low (Diniz-Filho and Telles 2002).

Results

Heterozygosity and allelic richness were high in deer populations in both the North and South regions. Regions did not differ from one another in heterozygosity ($t= 0.737$, $df= 2$, $p= 0.538$) or allelic richness ($t= -0.157$, $df= 2$, $p= 0.889$) over time (Table 10).

Estimates of variance in allele frequencies among quadrats of deer (Fst) in the network of 23.3-km^2 quadrats were close to zero, and indicate that the size of quadrats may have exceeded the scale of spatial genetic structure present in both regions during each year (Diniz-Filho and Telles 2002). Fst values were effectively zero in South (-0.001, -0.001, and -0.001) and relatively low in North (0.004, -0.003, and 0.004) for

Table 10. Heterozygosity and allelic richness (±SE) for deer genotyped in the North and South regions during 1998, 1999, and 2000.

Region	Year	Observed Heterozygosity	Allelic Richness
North	1998	0.803 (0.023)	9.26 (0.06)
North	1999	0.771 (0.045)	8.99 (0.05)
North	2000	0.778 (0.032)	8.67 (0.03)
South	1998	0.760 (0.046)	9.16 (0.06)
South	1999	0.779 (0.041)	8.58 (0.06)
South	2000	0.778 (0.047)	9.33 (0.07)

1998, 1999, and 2000 respectively. The network of 2.6-km^2 quadrats may be a more biologically appropriate scale at which to characterize deer spatial genetic structure in DMU452.

The degree of genetic divergence among quadrats (Fst) as measured using the network of 2.6-km^2 quadrats was effectively zero in the South region and relatively low in the North region prior to the ban on artificial feeding (1998) (Table 11). The near zero Fst values imply that allele frequencies among quadrats of deer were relatively homogenous when artificial feeding was occurring. The degree of population structure as measured by variation in allele frequencies among quadrats of deer (Fst) was greater in both regions following the ban on artificial feeding (2000) (Table 11). Higher estimates of inter-quadrat variance in allele frequencies was observed among quadrats of deer in the North region relative to the South region both prior to and following changes in feeding regulations (Table 11). The greater variance in allele frequencies among quadrats of deer in the North region relative to the South region may be due to the North region's less intensive artificial feeding history.

There was a significant correlation between genetic and geographic distance among quadrats in the South region in 2000 and a marginal relationship among quadrats in the North region in 1998 as measured using the network of 23.3-km^2 quadrats of deer (Table 12). Correlograms depicted increased genetic differentiation between quadrats with increased geographic distance in the North region in all years, and in the South region in 2000 (Figure 9).

There was no significant relationship between genetic and geographic distance in either the North or South regions in 1998 as measured by the network of 2.6-km^2

Table 11. Measures of allelic variance within and among quadrats for the network of 2.6-km^2 quadrats for the North and South regions during 1998, 1999, and 2000.

Region	Year	Fis	Fst	Fit
North	1998	0.020	0.005	0.025
North	1999	0.025	0.010	0.034
North	2000	0.013	0.027	0.039
South	1998	0.050	0.001	0.050
South	1999	-0.166	-0.049	-0.223
South	2000	0.023	0.004	0.027

Table 12. Results of Mantel tests of correlations between genetic differentiation between quadrats and their geographic distance including results of permutation tests of significance and associated p-values for the network of 23.3-km^2 quadrats for the North and South regions during 1998, 1999, and 2000.

			# of iterations out of 1000		
Region	Year	Correlation	< Observed	> Observed	p-value
North	1998	0.115	909	91	0.092
North	1999	0.023	648	352	0.353
North	2000	0.061	797	203	0.204
South	1998	-0.168	6	994	0.994
South	1999	-0.081	115	885	0.885
South	2000	0.134	960	40	0.041

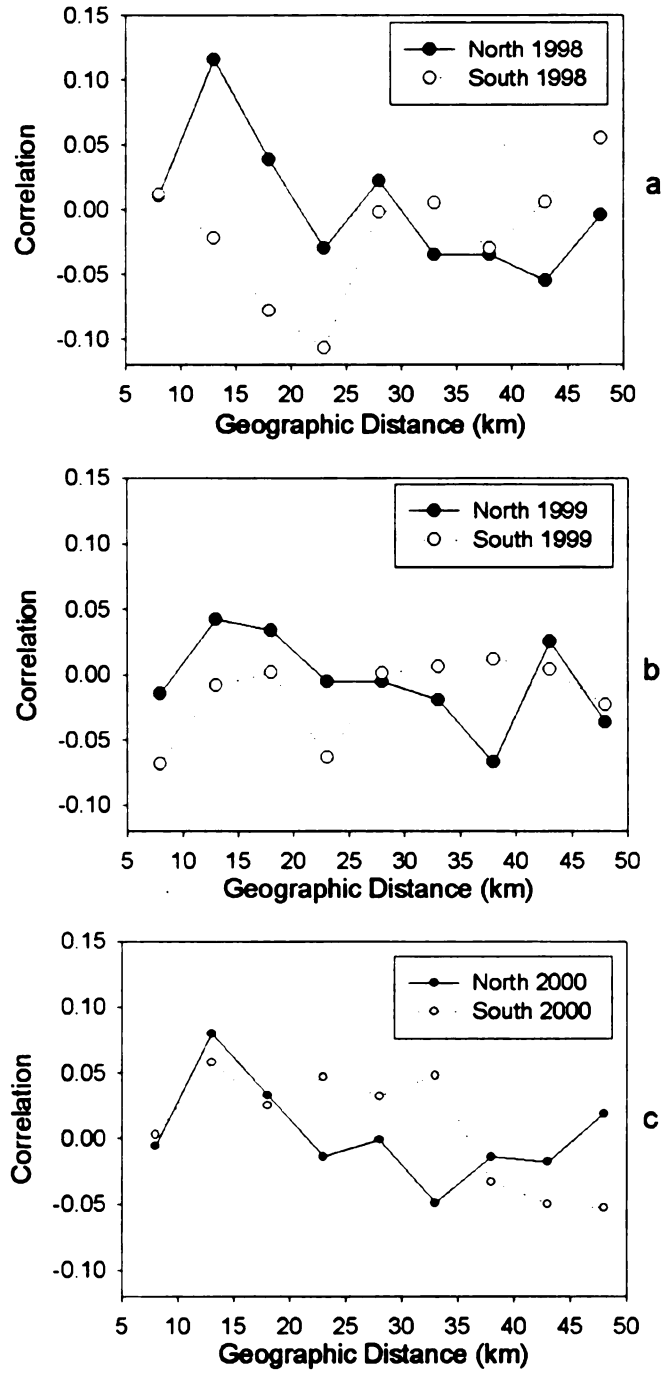

Figure 9. Correlation between quadrats of deer separated by different geographic distances in the North and South regions for the network of 23.3-km^2 quadrats in the year prior to the ban on artificial feeding (1998) (a), the year in which feeding was banned (1999) (b), and the year following the ban on feeding (2000).

quadrats of deer (Table 13). When artificial feeding was occurring, spatial genetic structuring was not detected in either region. There was, however, significant spatial autocorrelation in both regions in 2000 (Table 13). Following the ban on artificial feeding, both regions showed evidence of significant heterogeneity among quadrats of deer across space. The magnitude of spatial autocorrelation was higher in the North region than in the South region in all years (Table 13). A stronger signature of spatial genetic structuring in the North region relative to the South region can likely be explained by the North region's historically lower level of artificial feeding.

Correlograms of correlation coefficients at several geographic distances indicated the degree of genetic differentiation between quadrats of deer increased with increasing spatial distance in both the North and the South regions in 2000 relative to 1998 using the network of 2.6-km^2 quadrats (Figure 10). In addition, in 2000, the magnitude of genetic differentiation between quadrats of deer as a function of increasing geographic distance was greater among in the North region than in the South region (Figure 10). The geographic distance at which the autocorrelation becomes non-significant can be defined as the intercept of the correlogram (Diniz-Filho and Telles 2002), and lies between 13-18 km in each region for 2000. The intercept of a correlogram defines the minimum geographic distance at which samples are independent or the diameter of a genetic patch (Diniz-Filho and Telles 2002). The intercepts of the correlograms for both regions are consistent with telemetry data that indicates that the average maximum distance of deer movement in DMU452 is approximately 16 km (Garner 2001).

Table 13. Results of Mantel tests of correlations between genetic differentiation between quadrats and their geographic distance including results of permutation tests of significance and associated p-values for the network of 2.6-km^2 quadrats for the North and South regions during 1998, 1999, and 2000.

			# of iterations out of 1000		
Region	Year	Correlation	< Observed	> Observed	p-value
North	1998	0.023	805	195	0.196
North	1999	0.007	625	375	0.376
North	2000	0.087	996	4	0.005
South	1998	-0.008	313	687	0.687
South	1999	-0.009	366	634	0.634
South	2000	0.061	992	8	0.009

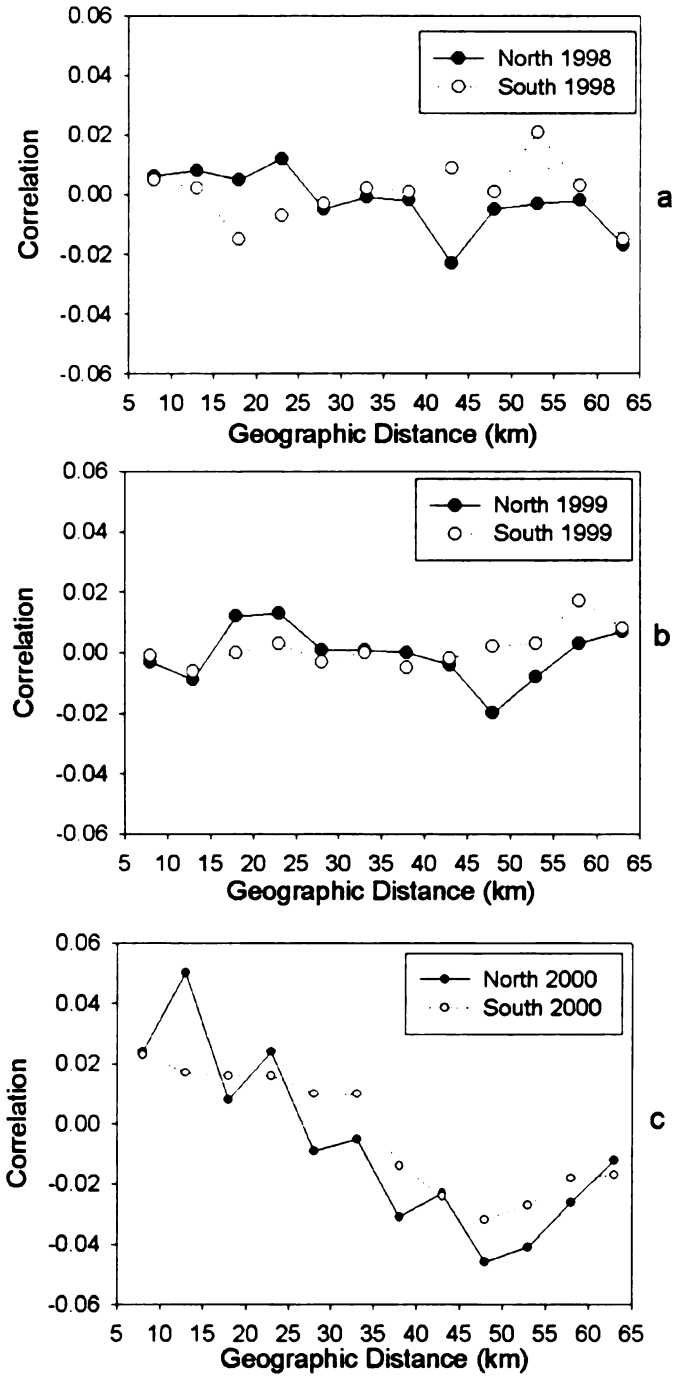

Figure 10. Correlation between quadrats of deer separated by different geographic distances in the North and South regions for the network of 2.6-km^2 quadrats in the year prior to the ban on artificial feeding (1998) (a), the year in which feeding was banned (1999) (b), and the year following the ban on feeding (2000).

Discussion

Prior to the ban on artificial feeding, there was no evidence of genetic divergence among quadrats of deer (Fst) or significant correlation between genetic differentiation and geographic distance between quadrats of deer in either the North or South regions. The absence of significant spatial autocorrelation in 1998 may be explained by the aggregation of multiple matrilines at artificial feeding sites. Following the ban on artificial feeding, there was a demonstrable increase in the degree population genetic structure as measured by variance among quadrats in allele frequencies (Fst) in both the North and South regions. Additionally, there were significant correlations between the degree to which quadrats of deer were genetically differentiated from one another and their geographic distance in both regions. The detection of significant spatial genetic structure in 2000 is likely the result of a greater dispersion of matrilines across space. Increasing genetic differentiation between groups of deer with increasing geographic distance in both regions suggests that there is likely limited gene flow among quadrats of deer as they become increasingly separated in space as expected in an isolation by distance model (Purdue et al. 2000).

The degree to which quadrats of deer were genetically differentiated from one another was greater in the North region relative to the South region for all time periods. The greater spatial genetic structuring found in the North region relative to the South region is likely due to the North region's historically lower level of artificial feeding. The magnitude at which artificial feeding was practiced in the South region likely served to bring larger numbers of individuals together at artificial feeding sites than did artificial feeding sites in the North region. The aggregation of a larger number of individuals from

a variety of different matrilines in the South region likely resulted in a greater degree of homogenization of allele frequencies than occurred in the North region.

In addition, telemetry studies of deer located in the South region conducted by Garner (2001) documented that when artificial feeding was occurring, deer moved extremely short distances relative to movement distances documented in deer elsewhere in the United States (Garner 2001). The lesser degree of spatial genetic structuring in the South region relative to the North region may be a function of the extremely short movement distances for deer in the South region when food was abundant. Turnover of the population and recruitment into the population would be expected to result in increasing spatial genetic structuring in the South region in the years following the ban on artificial feeding as more traditional movement behavior resumes in the region.

Data from 1999, the year in which artificial feeding was banned, for both regions and both networks were somewhat unusual compared with results from 1998 and 2000 (Figure 9, 10) (Table 12, 13). It is possible that the lack of food at locations where deer found it for several decades may have resulted in somewhat unusual movement in 1999. Deer show considerable fidelity to feeding sites, and it may take population turnover and recruitment for all individuals to abandon sites where food piles no longer occur.

Social structuring in natural populations can impede the movement of genes among breeding groups, serving to partition population genetic variation (Chesser 1991a,b, Sugg et al. 1996). For animals in which females remain in natal areas near relatives, genetic differences will accrue among spatially segregated matrilines (Chesser 1991a, Matthews & Porter 1993). Male breeding structure can either accentuate levels of divergence or homogenize gene frequencies across group structured populations (Chesser

1991a,b, Matthews & Porter 1993). Gene correlations among individuals within social lineages (θ) are enhanced when a single or a few males breed within a lineage (Chesser 1991a). However, when numerous males mate within a social lineage or when males mate with females from several lineages, coancestry among individuals within a lineage (θ) decreases relative to coancestry among individuals from different lineages (α) serving to homogenize population gene frequencies (Chesser 1991a). Future studies of animals recruited into the deer population after the ban on artificial feeding may be useful in addressing the impact of the artificial feeding ban and reduced density on the deer population's mating system.

Numerous studies have documented the presence of spatial genetic structure in white-tailed deer populations (Cronin at el. 1991, Matthew and Porter 1993, Scribner 1993, Purdue et al. 2000). Few studies, however, have characterized the degree of homogenization in allele frequencies among quadrats of deer across space as was documented here when artificial feeding was occurring. Several studies have documented that anthropogenic actions such as the method and intensity of harvest and translocations can alter spatial genetic structuring in deer populations (Leberg 1990, Scribner 1993, Scribner et al. 1997, Leberg and Ellsworth 1999). Leberg and Ellsworth (1999) found that animal translocations reduced the degree to which populations were genetically differentiated as a function of their geographic distance from one another. Scribner et al. (1997) found significant temporal variation in microgeographic gene frequencies that could likely explained by high rates of population turnover, young age structure, and method of harvest affecting local breeding structure. The results presented here are the first to document the extreme homogenization of allele frequencies among groups of deer

when artificial feeding was occurring, and the rapid emergence of spatial heterogeneity in allele frequencies among quadrats of deer following the ban on artificial feeding.

Analyses of the degree to which natural populations are spatially genetically structured can be used to make inferences regarding the effects of management actions on movement patterns and breeding behaviors that would be difficult to determine using traditional methods. Changes in deer spatial genetic structure coincident with changes in management can also have important implications with regard to disease transmission in wildlife populations. Disease transmission will be affected by the spatial distribution of infected individuals (Fromont et al. 1998a,b, Tully et al. 1999, Fulford et al. 2002). Clusters of genetically similar groups of deer in space indicate that there is spatial genetic structuring in DMU452 that likely limits the movement of TB across genetically differentiated groups. Knowledge of the magnitude of spatial genetic structure and the distance of gene flow as measured by spatial variation in allele frequencies can be incorporated into management strategies aimed at delineating hotspots of TB, targeting regions for management actions such as intensive density reductions, and predicting the future transmission and distribution of bovine tuberculosis.

CHAPTER 4

A SIMPLE MODEL HIGHLIGHTING THE IMPORTANCE OF SOCIAL STRUCTURE IN THE MAINTENANCE OF BOVINE TUBERCULOSIS IN FREE-RANGING WHITE-TAILED DEER

Transmission dynamics drive interactions between host and pathogen populations (Begon et al. 1999). Host-pathogen models are used to design management strategies for disease control (McCallum et al. 2001). Traditionally, in the majority of both empirical and theoretical studies, disease transmission dynamics have been assumed to be density dependent (mass action) (Anderson and May 1992, Grenfell and Dobson 1995, Begon et al. 1999, McCallum et al. 2001). In density dependent models of disease transmission contacts among infected and susceptible individuals are assumed to be random and to increase in proportion to population size (Begon et al. 1999). Culling in order to reduce population density is a common management strategy for controlling disease based on density dependent models (McCallum et al. 2001). This policy will fail, however, if disease transmission is not solely a function of host density (McCallum et al. 2001).

Alternatively, disease transmission dynamics may be frequency dependent where the number of contacts among infected and susceptible individuals are fixed and independent of population size (Begon et al. 1999). In addition, the incidence of infection is often patchily distributed, where infected individuals are likely to be closer to other infected individuals than expected under random mixing which is the typically assumed model of disease transmission (McCallum et al. 2000). The manner in which transmission dynamics are modeled will have profound effects on the success of management actions aimed at eliminating disease transmission (Dobson and Meagher 1996).

Most zoonotic pathogens are endemic, always present at some low level, in wildlife populations (Childs et al.1998, Begon et al. 1999). Most empirical studies of disease transmission in wildlife, however, have been conducted opportunistically in response to epidemic outbreaks that generally occur over short time periods (Begon et al. 1999). Endemic diseases are rarely easily eradiated, and ecological changes can promote their amplification to epidemic levels (increased incidence) (Childs et al. 1998). As such, it is important to understand the key mechanisms of disease transmission to devise effective control measures (Childs et al. 1998).

The white-tailed deer (*Odocoileus virginianus*) population in the lower peninsula of northeast Michigan (MI) (DMU452) is infected with bovine tuberculosis (*Mycobacterium bovis*) (TB). The management actions to eliminate TB from free-ranging deer are based upon a density dependent model of disease transmission. Management is focused on minimizing contacts and crowding among deer at feeding sites and decreasing population density. One major action taken in order to decrease the transmission of TB was to ban artificial feeding. Artificial feeding results in large aggregations of deer for prolonged periods of time. It is likely that the probability of encountering food contaminated with *M. bovis* at artificial feeding sites is greater than in a deer population foraging on natural foods. In the absence of feeding sites, it was hypothesized that deer would disperse in search of food, and contacts and crowding among deer would decrease thereby greatly reducing TB transmission.

The second major management action was to increase the antlerless (female) deer harvest to decrease deer population density. Increased hunting, especially of female deer, was intended to reduce deer numbers to levels that could be supported by natural habitat.

It was hypothesized that the combination of the management strategies described above would reduce TB transmission. When transmission decreases to some threshold level, more deer infected with bovine TB will die each year than would become infected, and disease prevalence would be expected to decline. Management goals were to decrease disease prevalence to less than one percent by the fall of 2003, and to have TB eliminated from the wild deer herd by the fall of 2010 (MDNR TB Activity Report 1997).

The ban on artificial feeding along with increased hunting are likely to result in decreased transmission of TB among deer by eliminating the risk of infection due to contacts among individuals at feed sites. However, the matrilineal social structure and female philopatry of white-tailed deer populations results in frequent interactions among related individuals and rarer interaction among non-relatives (Hawkins and Klimstra 1970, Marchinton and Hirth 1984). The artificial feeding ban and reduction in population size do not specifically address the non-random probability of infection with TB as a function of white-tailed deer ecology. Specifically, deer that are closely related to an infected deer have a higher risk of TB infection than do nonrelatives.

An understanding of the role of deer ecology in disease transmission is necessary to evaluate whether transmission dynamics more closely resemble density dependent or frequency dependent processes, and whether current management actions will eliminate the disease. Numerous recent studies have demonstrated that wildlife (host) ecology plays a key role in disease transmission (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). As was demonstrated in Chapter 2 of this dissertation, TB-positive deer were more closely related than were non-infected deer both prior to and following management efforts to

decrease contacts over food and to decrease density in northeast MI. Clearly, the probability of infection with TB is non-random such that deer that are closely related to an infected deer have a higher risk of TB infection than do nonrelatives suggesting that genealogical relationships play a key role in transmission of TB. In order to effectively control TB, all mechanisms of TB transmission must be accounted for in management actions.

McCarty and Miller (1998) were the first to model TB transmission among deer in northeast Michigan. Interactions among infected and susceptible individuals were assumed to be random and to change as a function of population size (McCarty and Miller 1998). In their model, all individuals had an equal probability of becoming infected with TB. McCarty and Miller (1998) described 3 factors that determined the probability that an individual would become infected: the number of infected individuals in the population, the number of contacts the individual made, and the total population size. The model predicted that TB would increase substantially over time regardless of management efforts to reduce disease transmission (McCarty and Miller 1998).

The effectiveness of management actions to reduce population density and artificial feeding on TB prevalence was modeled (Hickling 2002). Because of the mass action model's success in understanding TB transmission in New Zealand brushtail possums (*Trichosurus vulpecula*), a similar model was used to examine TB transmission among deer (Hickling 2002). A regression model in the region of highest TB prevalence predicted that reduction in herd density by one-third along with reduction in artificial feeding by 75% would decrease TB prevalence to 0.5% after 10 years (Hickling 2002). The model indicated that under current management, complete eradication of TB was

unlikely. The model did predict, however, that further reductions in either deer density (an additional 40-50%) or artificial feeding alone could potentially result in the elimination of TB (Hickling 2002).

The McCarty and Miller (1998) and Hickling (2002) models predict dramatically different trajectories for TB in deer in DMU452. Unfortunately, both models fail to incorporate characteristics of deer ecology that influence the probability of infection with TB. Results from Chapter 2 of this dissertation indicated that deer infected with TB were more closely related than were non-infected individuals both prior to and following efforts to reduce density and contacts among deer. TB-infected deer were more closely related than were non-infected deer within distances consistent with deer movement in the lower peninsula of northeast MI indicating that the probability of infection with TB is not random. Thus, unlike what has previously been modeled, TB transmission, facilitated through interactions among relatives within matrilines, is at least partially frequency dependent. Here, a model is presented highlighting interactions among relatives (within matrilines) as an important risk factor for TB infection.

Methods

STELLA modeling software (Costanza et al. 1998) was used to create a conceptual and simple quantitative model of TB transmission to evaluate whether TB would be eliminated from DMU452 over a 20 year period starting in 1998, the year prior to management actions taken to eradicate TB (Figure 11). The main purpose of the model was to explicitly incorporate TB transmission among relatives (non-random, frequency dependent), and separate it from random transmission among non-relatives (density

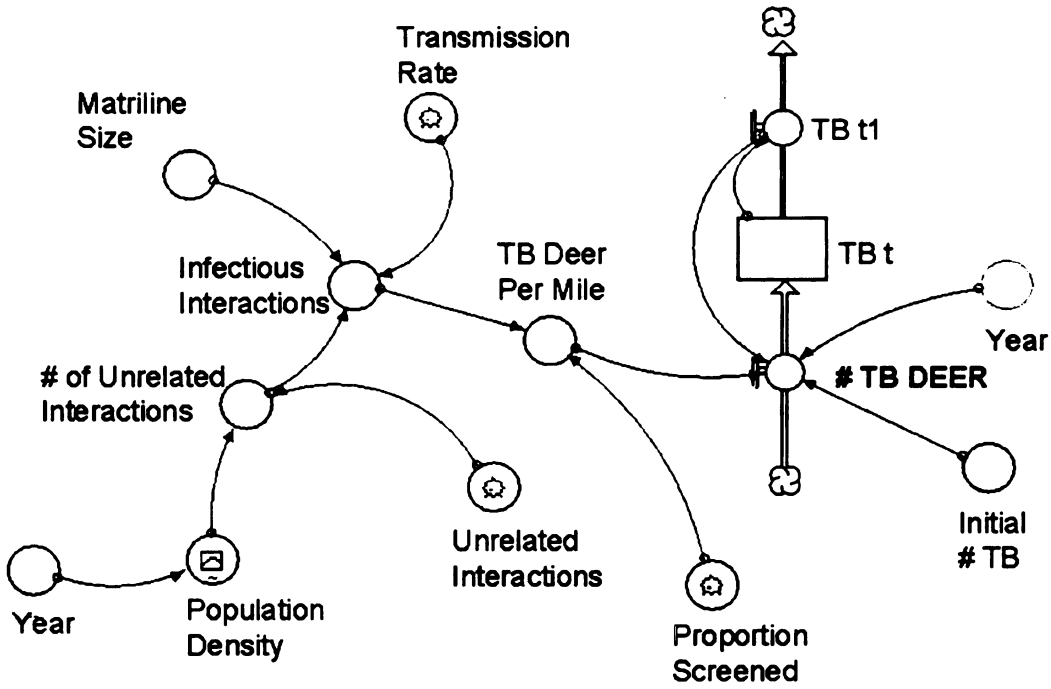

Figure 11. A conceptual model of TB transmission among deer in the northeast lower peninsula of Michigan.

dependent). Separation of density dependent and independent components was deemed appropriate based on results from Chapter 2 that indicated that TB-positive deer were more closely related than non-infected deer, implying that interactions and disease transmission within groups of related and non-related individuals must be different. The initial population density was set at 50 deer per square mile based on estimates of deer population density in 1998. Density was modeled to decline as predicted by the MDNR (Schmitt et al. 1997, Hickling 2002) (Table 14). The number of interactions among unrelated individuals was set at 15% of population density per square mile (Table 14). The number of interactions among unrelated individuals was set at 15% in order to make interactions among non-relatives nearly the same as the number of interactions among related individuals, size of matrilines, at the beginning of the model. The probability of interaction among non-related individuals was expected to decline following the ban on artificial feeding and as population density declined (Table 14).

Matriline size was parameterized based on previous studies of matrilineal behavior in which females have been shown to live in groups usually consisting of an adult doe, her yearlings and fawns, and often her older offspring as well as her sisters and their offspring (Hawkins and Klimstra 1970, Marchinton and Hirth 1984). Deer within matrilines have been observed to come into frequent contact with one another. Hawkins and Klimstra (1970) found that 87% of yearling females remained philopatric and were observed with other group members in greater than 60% of all observations. The average size of a deer matriline varies based on a number of characteristics including rate of population turnover, type of habitat, and age structure of the population. Matriline size

Table 14. Initial values of parameters included in the model of TB in deer in northeast Michigan.

Parameter	Value
Year	Counter (0,21)
Population Density per mi^2	Year 0-1= 50, 2= 41, 3-14= 32, 15-17= 18, 18-20= 12
Unrelated Interactions	Constant 15%
# of Unrelated Interactions	Population Density * Unrelated Interactions
Matriline Size	Constant 7
Transmission Rate	Constant 15%
# of Infectious Interactions	(# of Unrelated Interactions + Matriline Size) * Transmission Rate
Proportion Screened	Constant 25%
# of TB Deer Per Mile	# of Infectious Interactions * Proportion Screened
TB	TBt (t-dt) + (Deer TB – TBt1) * dt
TB t1	TBt
Initial # of TB Deer	58
# of TB Deer	If year = 0 then Initial # TB+ Deer else # of TB Deer Per Mile * (TBt + TBt1)

was modeled here to be a constant 7 deer assumed to be coming into frequent contact (Table 14).

The rate of disease transmission per contact is one of the most difficult components of the model to parameterize. A number of studies have been carried out to evaluate the efficacy of TB transmission among captive animals and from artificial feed (Whipple and Palmer 2000). Very little is known, however, about TB transmission resulting from face to face contacts among free-ranging deer. For this model, the probability of TB transmission among interacting deer was extrapolated from recent studies to be 15% of all contacts (Table 14) (Palmer et al. 1999).

Total infectious interactions was the sum of the number of interactions among unrelated individuals and matriline size multiplied by the transmission rate (Table 14). Not all deer that are infected with TB in DMU452 are detected because only harvested deer are screened for TB. A parameter called "Proportion Screened" was included in the model to account for the fact that only harvested deer, approximately 25% of the population, are screened for TB (Table 14) (Hickling 2002). The number of TB-positive deer detected per mile was a function of the number of infectious interactions multiplied by the proportion of deer screened for TB (Table 14). The total number of TB-positive deer was determined by multiplying the number of TB-positive deer per mile by the number of TB-positive deer in the previous year, initially 78 (Table 14) (O'Brien 2002).

Validation of this model was not possible because there were no pre-existing data regarding TB transmission in this or any other free-ranging cervid population in North America. The model was verified by comparing its prediction of TB in the first 4 years to empirical data (1998, 1999, 2000, and 2001) using a paired t-test.

The impacts of a variety of management scenarios on TB transmission were simulated by manipulating key model parameters. Specifically, the frequency of interaction among unrelated individuals was varied (0-25%) to reflect rates of contact among deer at different intensities of artificial feeding. Population density was manipulated to reflect different possible management goals. In addition, the matriline size was varied (0-7) to evaluate the impact of related interactions on the number of TB-positive deer.

Results

The output of the model indicated that the number of TB-positive deer declined as population density and interactions among unrelated deer declined (Table 15). Unlike the goals set by management to decrease disease prevalence to less than one percent by the fall of 2003, and to have the disease eliminated from wild deer by the fall of 2010 (MDNR TB Activity Report 1997), however, TB was not eliminated from the population (Table 15). The prediction of the model was that approximately 7 TB-positive deer would be identified in DMU452 in 2010. The ban on artificial feeding served to reduce interactions among unrelated individuals, but did not have an equivalent effect on interactions among related deer within matrilines. The continued higher incidence of TB among genetically similar individuals is responsible for the maintenance of TB in deer.

The model's prediction of the number of TB-positive deer in the first 4 years did not differ from the actual number of TB-positive deer detected in 1998-2001 ($t = -0.36$, $df = 3$, $p = 0.74$) (Table 16). The similarity of the model predictions to empirical data for the first 4 years helps verify the conceptual basis for parameterization of the model.

Table 15. Model predictions of population density, number of unrelated interactions, and number of TB-positive deer in northeast Michigan over 20 years. Years in which specific prevalence goals were set by management are 2003 (0.5%) and 2010 (0%).

Year	Population Density	# Unrelated Interactions	# TB Deer
0	50	10	78
1998	50	10	79.56
1999	41	8.2	72.56
2000	32	6.4	58.34
2001	32	6.4	46.9
2002	32	6.4	37.71
2003	**32**	**6.4**	**30.32**
2004	32	6.4	24.38
2005	32	6.4	19.6
2006	32	6.4	15.76
2007	32	6.4	12.67
2008	32	6.4	10.19
2009	32	6.4	8.19
2010	**32**	**6.4**	**6.58**
2011	32	6.4	5.29
2012	18	3.6	3.37
2013	18	3.6	2.14
2014	18	3.6	1.36
2015	12	2.4	0.77
2016	12	2.4	0.43
2017	12	2.4	0.24

Table 16. Number of TB-positive deer in northeast Michigan as predicted by the model and empirical data from 1998-2001.

Year	Model Prediction	Empirical Data
1 (1998)	79.56	78
2 (1999)	72.56	58
3 (2000)	58.34	53
4 (2001)	46.9	60

When parameters were manipulated to reflect alternative management scenarios, the number of TB-positive deer predicted in 2010 varied substantially. Further, more drastic reductions in population density and in interactions among unrelated individuals beyond current management goals resulted in fewer TB-positive deer (Table 17). It is doubtful, however, as to whether such extreme reductions in density or interaction rate among unrelated individuals could be accomplished. Reducing the size of matrilines, and thus interactions among related individuals, had the most rapid and greatest impact on reducing the number of TB-positive deer (Table 17).

Discussion

The model predicts that TB will decline following the ban on artificial feeding and decreased population density, but that TB will not be eliminated under current management actions. The model clearly illustrates that interactions among related individuals within matrilines play a key role in the maintenance of disease. Over time, interactions among unrelated individuals decline, but interactions among relatives within matrilines are constant and a function of matriline size. These interactions among relatives are responsible for the continued maintenance of TB in the deer population.

Previous attempts to model TB transmission among deer in DMU452 did not explicitly account for transmission among related individuals (McCarty and Miller 1998, Hickling 2002). The model developed by McCarty and Miller (1998) predicted that TB would increase substantially over time regardless of management efforts to reduce disease transmission. Empirical data of TB prevalence in DMU452 in the years following the development of the McCarty and Miller (1998) model indicated that TB prevalence

Table 17. Number of TB-positive deer in northeast Michigan in 2010 when initial values of parameters were varied.

Parameter Manipulation	# of TB Deer
Management Goal	0
Model	6.6
Unrelated Interactions 10% of population density	0.17
Unrelated Interactions 5% of population density	0.17
Unrelated Interactions 0% of population density	0
Unrelated Interactions 25% of population density	114.1
Population Density 18 per mi^2	3.3
Population Density 10 per mi^2	2.8
Population Density 5 per mi^2	2.5
Population Density 50 per mi^2	100.9
Matriline Size 4	0.27
Matriline Size 2	0.02
Matriline Size 1	0

has not increased (Hickling 2002). A regression model in the area of highest TB prevalence predicted that a one-third reduction in herd density along with a 75% reduction in artificial feeding would result in a TB prevalence of 0.5% after 10 or more years (Hickling 2002). The model predicted that further reductions in deer density (up to 40-50%) or artificial feeding could potentially result in an elimination of TB (Hickling 2002).

The density dependent Hickling model was based upon the success of density reductions in controlling TB in brushtail possums. The social structure of possums, however, is quite different from white-tailed deer. Brushtail possums are basically solitary animals (Nowak 1999). At high population densities individuals share dens and home ranges overlap, but at low population densities possums become solitary, territorial, and maintain discrete home ranges (Nowak 1999). White-tailed deer are highly social animals in which females are philopatric and live in matrilines while males live in buck groups for part of the year (Hawkins and Klimstra 1970, Marchinton and Hirth 1984). In addition, data from Chapter 2 clearly demonstrates that deer that are related to TB-infected deer are more likely to be infected with TB than are nonrelatives. Therefore, and as illustrated in the model presented here, manipulation of artificial feeding and overall population density are unlikely to result in the elimination of TB. Instead, TB is likely to be maintained at low prevalence in the deer population due to transmission among individuals within matrilines.

The model presented here was intended to highlight that the risk of TB infection as a function of genetic relatedness to an infected deer plays an important role in the transmission and maintenance of TB. The concepts presented in this model could be

incorporated into the Hickling model to increase its accuracy and predictability for use as a management tool. The model presented here also highlights areas of further research whose results might suggest management actions that will increase the likelihood that TB will be eliminated. For example, the results from Chapter 2 that indicated that the probability of TB infection is not solely density dependent suggests that there may be a critical threshold of population density below which density no longer predicts TB transmission. Below this threshold, TB infection might occur predominantly within a few infected matrilines. In addition, the relative role of density dependence in TB transmission will depend upon how many individuals within a matriline are infected with TB at a given time. If no more than one individual within a matriline is infected in a given time period then a reduction in total herd density will increase the probability that TB will be eliminated from a matriline. If, however, numerous individuals within a matriline are infected at the same time, then reducing total deer population density is not likely to eliminate all infected matrilines. Rather, more site specific reductions targeted at TB-infected matrilines might be more effective.

Characteristics of host ecology are increasingly recognized as playing key roles in host-pathogen transmission dynamics (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). In order to eliminate disease from wildlife populations it is necessary to understand the role that host ecology may play in disease transmission. This model is based on data that indicated that even prior to management actions aimed at eliminating TB, there was a non-random risk of infection with TB such that deer that were closely related to an infected deer had a higher risk of TB infection than did nonrelatives. As a result of the artificial feeding ban, lower

population densities, and female philopatry, unrelated individuals come into contact less frequently, and the disparity in the risk of infection for relatives of TB-infected deer versus nonrelatives will increase. The results of the model presented here highlight the role that deer social structure plays in the transmission and maintenance of TB.

CONCLUSIONS

Genealogical Relationships and the Probability of TB Infection

In this dissertation, estimates of genealogical relationships were used to infer the role that white-tailed deer ecology plays in the risk of infection with bovine tuberculosis. TB-positive deer were more closely related than were non-infected deer over the entirety of DMU452 as well as at distances consistent with deer movement in the northeast lower peninsula of MI (Garner 2001). Relatedness among TB-positive deer was higher than among non-infected deer both before and after management actions aimed at eradicating TB indicating that artificial feeding is not the sole mechanism by which TB is transmitted. Results indicate that the probability of infection is non-random such that deer related to a TB-infected deer have a greater probability of infection than do nonrelatives. Deer's strong matrilineal structure together with the non-random probability of infection implies that the risk of TB infection is not solely a function of density. The ban on artificial feeding and the current level of density reduction alone are unlikely to eradicate TB. Management must also focus on deer social groups that likely facilitate the maintenance of disease. The high rate of contact that occurs among related individuals within matrilines relative to rates of contact among individuals from different matrilines is one hypothesis as to the mechanism by which TB is transmitted among relatives. Additional mechanisms by which related groups facilitate the maintenance of TB that must also be evaluated include the possibility of genetic susceptibility to TB and risk factors in the environment to which all members of a matriline might be exposed.

Microgeographic Genetic Structure in Deer

Analyses of wildlife spatial genetic structure can be used to infer the impact of management actions on social structure, movement ecology, and breeding behaviors that would be difficult to determine using traditional methods. Molecular markers were used to evaluate the impact of artificial feeding and high deer densities on deer spatial genetic structure. Prior to the artificial feeding ban and increased levels of harvest, there was no significant relationship between genetic differentiation between groups of deer and their geographic distance from one another. Artificial feeding and high deer densities homogenized spatial genetic structure in DMU452 likely due to the aggregation of multiple matrilines at feeding sites. Following changes in management, there was evidence of heterogeneity in allele frequencies among quadrats of deer as well as a significant relationship between genetic differentiation between quadrats of deer and their geographic distance from one another. The magnitude of genetic differentiation between groups of deer as a function of geographic distance is likely the result of white-tailed deer matrilineal structure, female philopatry, and site fidelity previously documented in observational and telemetry studies (Hawkins and Klimstra 1970, Marchinton and Hirth 1984). Changes in deer spatial genetic structure coincident with changes in management can have important implications with regard to disease transmission in wildlife populations. Disease transmission will be affected by the spatial distribution of infected individuals (Fromont et al. 1998a,b, Tully et al. 1999, Fulford et al. 2002). Clusters of

genetically similar groups of deer in space indicate that there is spatial genetic structuring in DMU452, and likely limited movement of TB across genetically differentiated groups.

Future Research Directions

The results presented in this dissertation generate a number of questions regarding TB transmission and spatial genetic structure in white-tailed deer that can be addressed by further research. Results presented here indicate that the probability of TB infection is non-random and not solely density dependent. One major issue arising from these results is the relative role of density in the maintenance of TB. The role of density will depend upon the size of matrilines in DMU452 and how many individuals within a matriline are infected with TB at a given time. Characterization of the variability of the pathogen, *Mycobacterium bovis*, in the deer population, specifically within and among related groups of deer, would help to clarify whether contacts among individuals within matrilines facilitate disease transmission. Investigation into genetic susceptibility for TB infection would also contribute to an understanding of the risks of infection.

Characterization of spatial genetic structure in deer in DMU452 can also be approached using mitochondrial DNA (mtDNA) that is maternally inherited. Because females are typically philopatric, spatial genetic structure as measured using mtDNA would be useful in more precisely defining matriarchal structure in DMU452. Future studies of animals recruited into the population after the ban on artificial feeding may be useful in addressing the impact of the artificial feeding ban and reduced density on the deer population's mating system. In addition, the relationship between genetic

differentiation between groups of deer and the characteristics of habitat separating them may be useful in spatial explicit models aimed at predicting the transmission and distribution of TB in northeast MI.

Contributions to Management

The results presented in this dissertation contribute to efforts aimed at understanding the transmission and maintenance of bovine tuberculosis in white-tailed deer. An incorporation of these results with the research from other scientists has the potential to lead to a more complete understanding of the mechanisms of TB transmission among white-tailed deer. Such understanding will aid managers in developing strategies to eliminate TB. Specifically, the non-random probability of infection with TB as a function of genetic similarity to TB-infected individuals and the spatial aggregation of genetically similar groups of deer can be incorporated into spatially explicit models of the transmission and distribution of TB. Knowledge of the degree of population genetic structuring as measured by spatial variation in allele frequencies can be used with data identifying hotspots of TB to target such regions for management actions such as intensive density reductions in hotspot areas. Targeted density reductions in hotspots will be more likely to affect matrilineal groups that serve as reservoirs of TB infection.

General Contributions

Zoonoses are of increasing importance to wildlife conservation and human health (Childs et al. 1998). Transmission dynamics are driven by interactions between host and pathogen populations (Begon et al. 1999). It has become increasingly recognized that wildlife ecology plays a key role in disease transmission (Boone et al. 1998, Mills and Childs 1998, Begon et al. 1999, Hazel et al. 2000, Escutenaire et al. 2002, Meeus et al. 2002). It has been demonstrated based upon estimates of genealogical relationships that a significant reservoir of TB is within related groups likely facilitated by deer's strong social structure. This is the first study of its kind to use genetics to infer the role of wildlife behavior and social structure in transmission dynamics between host and pathogen populations. It would have been impossible to directly observe the movement, behavior, and relationships of the entire white-tailed deer population in DMU452. With access to hunter-harvested deer, however, molecular markers enabled genealogical relationships among individuals at both macro and microgeographic scales to be rapidly estimated. Data clearly demonstrate the utility of using molecular markers and population genetic theory to infer the role of host ecology in the maintenance of disease in wildlife. Finally, a number of studies have documented that anthropogenic impacts such as the method and intensity of harvest and translocations can alter spatial genetic structuring in deer populations (Leberg 1990, Scribner 1993, Scribner et al. 1997, Leberg and Ellsworth 1999). This study, however, is the first to document a relative homogenization of allele frequencies across a deer population when artificial feeding was occurring.

APPENDICES

APPENDIX I: MICROSATELLITE LOCI SCREENED IN WHITE-TAILED DEER FROM DMU452

Locus	Amplified	Optimized	# of Alleles	Size Range	Reference
BL 25	yes	yes	6	170-190	Talbot et al. 1996
BL 42	yes	yes	11	236-252	Talbot et al. 1996
BM 203	yes	yes	17	200-230	Talbot et al. 1996
BM 888	no	na	na	na	Talbot et al. 1996
BM 4107 [a]	yes	yes	15	139-167	Talbot et al. 1996
BM 4208	yes	yes	15	140-180	Talbot et al. 1996
BM 5004	yes	yes	23	180-230	Talbot et al. 1996
BMC 1009	yes	no	na	~270	Talbot et al. 1996
BMI 861 (Y)	yes	no: amps in fems	na	140-150	Bishop et al. 1994
Cervid 1 [a]	yes	yes	21	161-199	DeWoody et al. 1995
Cervid 2 [a]	yes	yes	11	158-178	DeWoody et al. 1995
Cervid 3	yes	yes	13	160-210	DeWoody et al. 1995
Cervid 14	yes	no	na	~190	DeWoody et al. 1995
CRFA	yes	yes	na	230-260	Moore et al. 1992
CSN3	yes	yes	3	185-195	Bishop et al. 1994
ETH152 [a]	yes	yes	14	200-232	Talbot et al. 1996
IGF1 [b]	yes	yes	17	114-158	Kirkpatrick 1992
INRA 008 (Y)	no	na	na	na	Vaiman et al. 1994
INRA 057 (Y)	yes	yes	1	?	Vaiman et al. 1994
INRA 062 (Y)	yes	yes	1	?	Vaiman et al. 1994
INRA 124 (Y)	yes	~yes	1	smeary	Vaiman et al. 1994
INRA 126 (Y)	yes	yes	1	181	Vaiman et al. 1994
IRBP2	yes	yes	8	160-180	Bishop et al. 1994
MAF35	no	na	na	na	Swarbick et al. 1991
MAF65	yes	no	na	~230	Buchanan et al. 1992
MAF70	yes	yes	18	100-140	Buchanan and Crawford 1992
OarFCB193 [a]	yes	yes	14	93-125	Buchanan and Crawford 1993
OarFCB304	yes	yes	2	120-140	Buchanan and Crawford 1993
OBCAM [b]	yes	yes	10	194-214	Fries et al. 1993
RT 1	yes	yes	10	210-240	Wilson et al. 1997
RT 5	yes	no	na	~160	Wilson et al. 1997
RT 6	yes	no	na	~85	Wilson et al. 1997
RT 7	yes	yes	15	200-240	Wilson et al. 1997
RT 9 [b]	yes	yes	11	105-129	Wilson et al. 1997
RT 10	no	na	na	na	Wilson et al. 1997
RT 20 [a]	yes	yes	13	223-259	Wilson et al. 1997
RT 23 [a]	yes	yes	22	148-190	Wilson et al. 1997
RT 24 [a]	yes	yes	13	202-236	Wilson et al. 1997
RT 27 [a]	yes	yes	15	139-167	Wilson et al. 1997
RT 30	yes	yes	20	160-210	Wilson et al. 1997
SRCRSP-3	no	na	na	na	Arevalo et al. 1994
SRCRSP-5	yes	yes	4	144-180	Arevalo et al. 1994
SRCRSP-10 [a]	yes	yes	8	208-222	Bhebhe et al. 1994

[a]= used in Chapter 2, [b]= used in Chapter 3

APPENDIX II: PRIMER RECIPES FOR OPTIMIZED WHITE-TAILED DEER PRIMERS

(all microliters)	All RTs	Cervid 1	Cervid 2	BM4208, BM5004	ETH152, CSN, SRCRSP-5	OarFCB 193	SRCRSP-10	BM410 7	OBCAM, IRBP	IGF-1
Buffer (PCR2)	2.5	2.5	2.5 (Mg	2.5	2.5	2.5	2.5	2.5	2.5	2.5
2 mM dNTP	1.5	1	1	2	2.5	1	2	1.5	2	2
10 microM primer	0.4	0.625	0.625	1	1	1	1	1	1	0.5
25 mM MgCl2	0.5	0	0.75	2	1	0	0	1	1.5	3
taq	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
dH2O	16.9	17.45	16.7	13.7	14.2	16.7	15.7	15.2	14.2	13.7
DNA	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5

	MAF70, OarFCB304	Cervid 3	BM203	multiplex: IGF-1, OBCAM, RT9 or IGF-1, OBCAM, CRF	
Buffer (PCR2)	2.5	2.5	2.5	2.5	
2 mM dNTP	2.5	1	2	3.5	
10 microM primer	1	0.625	1	0.6	RT9= 1.0
25 mM MgCl2	1	1.5	1	1.5	
taq	0.3	0.3	0.3	0.4	
dH2O	14.2	15.95	14.7	11	
DNA	2.5	2.5	2.5	2.5	

PCR Conditions (Celcius)

Loci	Initial	Denature	Anneal	Extend	Time	Cycles
RTs, Cervid 3, SRCRSP-10, OBCAM, IRBP, IGF-	94 1 min	94	54	72	1 min	30
Cervid 1, Cervid 2	94 1 min	94	58	72	1 min	30
BL42, BM4208, OarFCB304	94 1 min	94	50	72	30 sec	30
BM203, BM4107, BL25, CSN	94 1 min	94	48	72	30 sec	30
OarFCB193	94 1 min	94	56	72	30 sec	30
ETH152, MAF70	94 1 min	94	52	72	30 sec	30
BM5004	94 1 min	94	54	72	30 sec	30

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