2009

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Recommended Citation

Kewin, Jenna M., "Genetic Diversity, Fitness and Mating Behaviour in Song Sparrows (Melospiza melodia) (" (2009). *Digitized Theses*. 3917.
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Genetic Diversity, Fitness and Mating Behaviour in Song Sparrows (*Melospiza melodia*)

(Spine Title: Genetic Diversity, Fitness and Behaviour in Song Sparrows)

(Thesis Format: Integrated-Article)

By

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Understanding the evolutionary forces that maintain genetic diversity in natural populations is a major goal in behavioural and evolutionary ecology. I investigated the relationships between individual genetic diversity and pairwise genetic similarity (as measured by 17 microsatellite loci), mating strategies, parental investment, and overwinter survivorship, in song sparrows *Melospiza melodia.* Genetic similarity of socially mated pairs was not significantly different from random expectations, but females were more likely to produce extra-pair offspring when their social mate was genetically similar. Highly heterozygous males fed their nestlings significantly more often than did their less diverse counterparts, although this pattern was not observed in females. Interestingly, the high parental investment by genetically diverse males may come at a cost, as I discovered a highly significant negative relationship between male heterozygosity and overwinter survivorship. Collectively these findings suggest that trade-offs between current and future reproductive investment may temper the fitness advantages of genetic diversity.

Keywords

Heterozygosity-fitness correlations, mate choice, genetic compatibility, parental investment, song sparrow, *Melospiza melodia,* global effects, local effects, indirect benefits, microsatellite

Co-authorship

My supervisor, Dr. Elizabeth MacDougall-Shackleton will be given co-authorship on any publications that result from this thesis.

Acknowledgements

I would like to thank my supervisor, Dr. Beth MacDougall-Shackleton, for her patience in answering my incessant questions and her continued passion for science. Her ability to balance a thriving scientific career and a family, and still make it out to all the Mac-Shack celebrations is inspiring.

Dr. Bryan Neff and Dr. André Lachance, my advisors, provided me with valuable insights and support throughout my degree. I was lucky enough to learn from them in committee meetings, classes and even in the field or at the Grad Club. My labmates made me want to come into the office, lab, or field every morning, and truly made my graduate experience fantastic. Thanks to Yanina Sarquis Adamson, Janet Lapierre, Dominique Potvin, James King, Ainsley Furlonger, Shawn Kubli and Tyne Baker for being there through all the work, and especially the lunch breaks. Thank you to Dr. Shawn Garner for his assistance in resampling my data.

The Queen's University Biology Station not only made my field research possible, but also introduced me to field work adventures and great friends. Thanks to the University of Western Ontario for the opportunity to study in such a great environment.

To my family, thanks for tuning into the scandalous lives of song sparrows, your interest and support in whatever I pursue is what makes it all possible. Richard, thanks for always believing I could do it, no matter how hard I tried to convince you otherwise.

Table of Contents

List of Figures

List of Tables

List of Appendices

Chapter 1: General Introduction

The World Conservation Union (IUCN) identified genetic variation as a source of biodiversity that merits conservation efforts (McNeely et al. 1990). This is both due to the necessity of genetic variation for evolution, and to the associations found between genetic diversity and fitness (Reed and Frankham 2003). The lower individual genetic diversity associated with matings between relatives has been shown to cause decreased individual fitness and population health in a wide range of taxa (Tregenza and Wedell 2000). This phenomenon, known as inbreeding depression, has been studied across a variety of discliplines due to the implications for medicine, agriculture, conservation and evolution (Slate et al. 2004). Despite the importance of this field, our understanding of how individual genetic diversity relates to fitness is still weak, and requires further study (Chapman et al. 2009).

Population genetic diversity has been used as a measure of viability and adaptability (Reusch et al. 2005; Bazin et al. 2006) and individual genetic diversity has been positively associated with a variety of measures of fitness across taxa. Heterozygosity-fitness correlations (HFCs) often stem from the advantages of avoiding inbreeding depression (Tregenza and Wedell 2000). Inbreeding usually depresses fitness by unmasking deleterious recessive alleles but can also decrease fitness when heterozygosity itself is advantageous (Tregenza and Wedell 2000). Inbreeding depression is felt through decreased survival (Hass 1989; Alberts and Altman 1995; Keller 1998; Daniels and Walter 2000; Grant et al. 2001) and reproductive success (Rosenfield and Bielefeldt 1992; Bensch et al. 1994; Laikre et al. 1997; Slate et al. 2000) across taxa (reviewed in Keller and Waller 2002). With such severe costs of inbreeding in these contexts, genetically diverse individuals fare better.

While the above examples of HFCs are due to overall benefits of diversity across the genome (global effects), there is also strong evidence for heterozygote advantage at specific loci. The best examples of local effects of HFCs come from the major histocompatibility complex (MHC), a gene family involved in vertebrate immune function. Diversity at this complex has been associated with increased disease resistance (Arkush et al. 2002), juvenile survival and low parasitism (Paterson et al. 1998) and fecundity (Sauermann et al. 2001). The mechanisms behind these associations are well understood due to substantial research on MHC structure and function (Bernatchez and Landry 2003). There is substantial debate surrounding the ubiquity of global versus local effects of HFCs in wild populations, and to date there is empirical evidence for both (Tregenza and Wedell 2000; Lieutenant-Gosselin and Bernatchez 2006; Chapman et al. 2009; Szulkin et al. 2010).

The reduced fitness of highly inbred individuals or homozygotes at specific loci is well accepted although large outbred populations often demonstrate HFCs as well. This has generated substantial debate in recent literature, because mechanisms behind these HFCs are often poorly understood (Coltman and Slate 2003; Balloux et al. 2004; Dewoody and Dewoody 2005; Chapman et al. 2009; Szulkin et al. 2010). Major criticisms include publication bias for significant HFCs (Coltman and Slate 2003) even when this favors small sample sizes and few loci (Balloux et al. 2004). Using more genetic markers and studying life history traits that should be under directional selection at many loci would greatly enhance our knowledge of HFCs (Szulkin et al. 2010) especially in wild, outbred populations.

If there are benefits to individual genetic diversity, individuals that choose mates that are highly dissimilar should produce more genetically diverse, and thus more fit offspring. This genetic compatibility hypothesis has dramatically challenged our view of mate choice and genetic benefits, because it is the interaction between parental genotypes, rather than the action of an allele, that can yield benefits (Mays et al. 2008). In traditional good genes models of mate choice, female preference should converge on a specific phenotype, whereas in a genetic compatibility model female choice is predicted to be self-referential, and thus differ based on their individual genetic makeup (Mays et al. 2008). This incongruent female choice model fits with many socially monogamous mating systems that do not have high variance in male reproductive success. Advances in molecular techniques have made studies of mate choice for genetic compatibility possible (Zeh and Zeh 2003) but there is still much to learn. Much of our understanding of mate choice for genetic compatibility comes from studies of extra-pair mating behaviour, where no direct benefits are provided (Tregenza and Wedell 2000). It is important to recognize however, that good gene and compatible gene theories of mate choice are not mutually exclusive, and likely interact together in most populations (Zeh and Zeh 2003; Mays and Hill 2004; Neff and Pitcher 2005).

Mate choice operates primarily on the more competitive sex, and in many cases is assumed to be female driven. When both sexes invest highly in parental care, however, mutual mate choice is often expected (Johnstone et al. 1995). There are opportunities for mate choice throughout the breeding cycle, and evidence for compatible genes choice has been demonstrated at each stage (Neff and Pitcher 2005). Female sticklebacks (*Gasterosteus aculeatus*), choose MHC dissimilar males (Aeschlimann et al. 2003). Similarly, young female savannah sparrows (*Passerculus sandwichensis*), avoid MHC similar social mates, and MHC similarity in social pairs predicts production of extra-pair offspring (Freeman-Gallant et al. 2003). These examples of precopulatory mate choice suggest active choice. Mechanisms for this choice could be based on olfactory cues, as is the case for fish and mammals. These cues allow individuals to discriminate against mating with kin however; olfaction may be less developed in birds (Mays et al. 2008, but see Steiger et al. 2008). Even if olfactory cues are insufficient for birds to avoid mating with kin, there is evidence that song could play a role in kin detection in some species. Variation in calls allows the long-tailed tit (*Aegithalos caudatus*), to identify kin (Hatchwell et al. 2001). In song sparrows (*Melospiza melodia*), males singing local sounding songs are more genetically similar to the population than those singing unique songs (Stewart and MacDougall-Shackleton 2008). Postcopulatory, or cryptic, mate choice is well characterized in the red jungle fowl (*Gallus gallus*), where females eject more sperm from more genetically similar males (Pizzari et al. 2004) and males invest a greater volume of sperm in MHC dissimilar females (Gillingham et al. 2009). Finally, parents can choose to invest preferentially in some offspring (Burley

1988). In the context of mate choice for genetic compatibility, parents could bias care toward more genetically diverse offspring as demonstrated in song sparrows (*M. melodia,* Potvin and MacDougall-Shackleton 2009). Each stage of the breeding cycle also shows evidence of mate choice for good genes, and together, good and compatible genes contribute to overall genetic quality (Neff and Pitcher 2005).

Many species avoid the costs of inbreeding simply through their natural history; dispersal or delayed maturation makes inbreeding unlikely in many taxa, while other species avoid inbreeding depression through disassortative mate choice (Pusey and Wolf 1996). Reviews of mate choice for compatibility emphasize that benefits of avoiding inbreeding are likely to be highly context dependent. Neff and Pitcher (2005) focus on evolutionary cycles between choice for good genes and compatible genes and Mays et al. (2008) concentrate on behaviourally plastic variation in mate choice among individuals. Kempenaers (2007) finds that most support for genetic compatibility mate choice comes from extra-pair mating systems. Three widespread patterns support this idea: 1) social parental genetic similarity predicts level of extra-pair paternity, 2) extra-pair sires are more dissimilar than social fathers, and 3) extra-pair offspring are more genetically diverse than within-pair offspring. Each of these hypotheses supports mate choice for genetic compatibility, and many examples are found in avian mating systems (Kempenaers 2007).

My thesis focuses on a songbird that has provided many insights on HFCs and mating strategies. In a nonmigratory, insular, west coast population of song sparrows (*M. melodia*), cell-mediated immunity, song repertoire size, reproductive success and nestling survivorship decline with inbreeding (Keller 1998; Reid et al. 2005a, b, 2007). Despite this strong evidence for inbreeding depression, there was no support for inbreeding avoidance in this population (Keller and Arcese 1998). In contrast, our wild population of eastern song sparrows (*M. melodia melodia*) is migratory, philopatric, and presumably outbred, breeding north of Kingston, ON. This population has been monitored for several years. Both parents provide a high level of parental care. Genetic diversity is weakly correlated with song repertoire size, a sexually selected trait, and negatively correlated with severity of parasitic infection (Pfaff et al. 2007; Singh 2007). Unlike in the island population, however, genetic diversity in this population is unrelated to cell-mediated immunity (E. MacDougall-Shackleton and J. Kewin, unpublished data). Offspring of genetically dissimilar parents are fed more frequently and grow faster than offspring of similar parents (Potvin and MacDougall-Shackleton 2009) and extra-pair young are more heterozygous than within-pair young (Potvin 2008). Taken together, these studies suggest that genetic diversity is important to some aspects of fitness in small and large populations of song sparrows.

In this thesis I examine the relationships between individual genetic diversity, survivorship, and reproductive success, and how these relationships influence mating behaviour. I have built on previous work (Pfaff et al. 2007; Singh 2007; Potvin 2008) by more than doubling the number of microsatellite loci used to assess genetic diversity (as emphasized by Balloux et al. 2004; Chapman et al. 2009), and evaluating several new aspects of survivorship and reproductive success in nestling and adult song sparrows. In Chapter 2, I test the hypothesis that individual genetic diversity predicts survivorship in nestlings and adults by comparing genetic diversity to bacterial killing ability, adult overwinter survival, and nestling growth rates. I also test the hypothesis that individual genetic diversity is related to reproductive success by studying clutch size, within nest paternity, and investment in offspring, as measured by nest visits. In Chapter 2, I separate sexes when relationships between genetic diversity and fitness are different for males and females. In Chapter 3, I test whether song sparrows are mating randomly with respect to genetic similarity in their social or extra-pair mates. I also assess whether a social pair's genetic similarity predicts their clutch size, paternity, or investment in their offspring. Through this project, I hope to contribute to the growing body of literature about how genetic diversity shapes fitness and mating behaviour in wild, outbred populations.

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Chapter 2: Heterozygosity-fitness correlations in song sparrows (*Melospiza melodia*)

2.1 Introduction

The importance of genetic variation to the viability of populations and to the fitness of individuals is of great interest from both theoretical and applied perspectives. At the population level, genetic diversity is a key determinant of a population's ability to respond to environmental changes (Reusch et al. 2005) and lack of genetic variation is often an indicator of a threatened or endangered population (Bazin et al. 2006). At the individual level, genetic diversity (often quantified as multilocus heterozygosity) is positively associated with various aspects of fitness in many taxa. Low genetic diversity has been implicated in susceptibility to parasites in Soay sheep (*Ovis aries,* Coltman et al. 1999), reduced seed set in montane herbs (*Ipomopsis aggregate,* Waser and Price 1991), decreased fertility in fruit flies (*Drosophila nigrospiracula,* Markow 1997), reduced hatching success in great reed warblers (*Acrocephalus arundinaceus,* Bensch et al. 1994), immunodeficiency in cheetah (*Acinonyx jubatus,* O'Brien 1994), and hypertension in humans (Rudan et al. 2003). Across 99 species of wild birds, moreover, hatching success is dramatically reduced in inbred relative to outbred populations (Spottiswoode and Møller 2004). Thus, understanding the processes that maintain genetic diversity and how it can relate to fitness and behaviour has implications for conservation as well as evolution and ecology.

As reviewed above, heterozygosity-fitness correlations have been observed across taxa in a wide variety of fitness-related traits. Recent theory suggests that the most informative fitness traits to examine for HFCs are likely to be those that are highly polygenic and exhibit directional dominance (Szulkin et al. 2010). Thus, much attention has been focused on life history traits, which are directly related to fitness and also tend to have more variation due to mutation than morphological traits (Houle et al. 1996). However, life history traits and strategies often involve complex trade-offs between different aspects of fitness. Studies attempting to quantify fitness should therefore consider several indicators of survivorship and reproductive success in their assessments (Neff and Pitcher 2005).

One important mechanism which may maintain genetic diversity within natural populations is that genetically diverse individuals have higher survivorship than their less diverse counterparts. Studies of wild animal populations often focus on juvenile survival or recruitment and adult immune function as proxies for survival. Individual genetic diversity has been positively associated with decreased susceptibility to pathogens and infections (Chinook salmon, *Oncorhynchus tshawawytscha,* Arkush et al. 2002; California sea lions, *Zalophus californianus,* Acevedo-Whitehouse et al. 2003; harbour seals, *Phoka vitulina,* Rijks et al. 2008) and parasites (bighorn sheep, *Ovis canadensis,* Luikart et al. 2008; Cuvier's gazelle, *Gazella cuvieri,* Cassinello et al. 2001). In other species such as the European eel (*Anguilla anguilla*), however, no relationship was found between heterozygosity and parasite infestation (Pujolar et al. 2009). Heterozygosity has also been positively associated with offspring survival in common shrews (*Sorex araneus,* Stockley et al. 1993), Soay sheep (*Ovis aries,* Coltman et al. 1999), alpine marmots (*Marmota marmot,* Cohas et al. 2009), and greater horseshoe bats, (*Rhinolophus ferrumequinum,* Rossiter et al. 2001), as well as birth weight and neonatal survival in red deer (*Cervus elaphus,* Coulson et al. 1998) and adult survival in threespine stickleback (*Gasterosteus aculeatus,* Lieutenant-Gosselin and Bernatchez 2006). These heterozygosity-fitness correlations (HFCs) represent some of the many examples of how genetic diversity can affect survival in the wild.

The second major mechanism whereby genetic diversity may affect fitness is a positive relationship between reproductive success and individual genetic diversity. Supporting this, genetic diversity is related to short-term mating success in damselflies (*Coenagrion scitulum,* Carchini et al. 2001), number of offspring in rhesus macaques (*Macaca mulatto,* Sauermann et al. 2001), and yearly reproductive success in brown bears (*Ursus arctos,* Zedrosser et al. 2007). In many cases, this positive relationship between genetic diversity and reproductive success is mediated by greater expression of sexually selected traits, and expressed only in males (reviewed in Brown 1997).

HFCs are especially well studied in birds. Low genetic diversity is associated with increased risk and intensity of parasitism in mountain white-crowned sparrows (*Zonotrichia leucophyrs oriantha,* MacDougall-Shackleton et al. 2005), decreased cell-mediated immunity in nestling bluethroats (*Luscinia svecica,* Fossøy et al. 2009) and decreased cell-mediated immunocompetence and pathogen resistance in house finches (*Carpodacus mexicanus,* Hawley et al. 2005). Heterozygosity is also related to recruitment and survival in great reed warblers (*Acrocephalus arundinaceus,* Hansson et al. 2001, 2004). Moreover, despite the widespread existence of trade-offs between survivorship and reproductive success (parasites and sexually selected traits, Folstad and Karter 1992; parasitism and reproductive effort, Norris et al. 1994; health status and reproductive effort, Ots and Horak 1996; immunocompetence and reproductive effort, Ardia 2005) there is also substantial support for HFCs in avian reproductive success as well. Most published examples of heterozygote advantage involve sexually selected traits expressed in males, such as song structure and territory size in subdesert mesite (*Monias bensch,* Seddon et al. 2004), song complexity in sedge warblers (*Acrocephalus schoenobaenus,* Marshall et al. 2003) and song sparrows (*Melospiza melodia,* Reid et al. 2005), and throat color in spotless starlings (*Sturnus unicolor,* Aparicio et al. 2001). Though less prevalent in the literature, there is some evidence of female-specific HFCs including clutch size in blue tits (*Cyanistes caeruleus,* Foerster et al. 2003), and great tits (*Parus major,* Tomiuk et al. 2006), and fledging success in wandering albatross (*Diomedea exulans,* Amos et al. 2001). In an island population of song sparrows, inbred females produce slow-growing offspring with relatively weak immune responses, suggesting that inbred females may be less capable of provisioning their young (Reid et al. 2003).

Whereas inbreeding depression in small and isolated populations is widely accepted, many of the studies reviewed above reported HFCs in large, presumably outbred, populations and have generated substantial controversy in the recent literature (Coltman and Slate 2003; Balloux et al. 2004; Chapman et al. 2009;

Szulkin et al. 2010). One major concern is that multilocus heterozygosity at marker loci may be a poor proxy for measuring inbreeding levels (Balloux et al. 2004; Dewoody and Dewoody 2005). Although this concern can be minimized through using very large numbers of loci and individuals, such requirements can be prohibitive to empiricists studying non-model organisms in the wild. Moreover, understanding the underlying genetic architecture of HFCs is essential to interpreting these relationships (Hansson and Westerberg 2002) so that mechanisms behind both expected and unexpected HFCs can be considered. Finally, when statistically significant relationships between genetic diversity and fitness are not detected, such negative results should likewise be reported and discussed, to reconcile the publication bias prevalent in this field (Balloux et al. 2004; Chapman et al. 2009; Szulkin et al. 2010).

The mechanisms responsible for observed correlations between heterozygosity and fitness depend upon the type (coding or non-coding) as well as the number of loci used to measure genetic diversity. Heterozygote advantage, or the non-additive genetic benefit of heterozygosity, has been shown both at coding loci (Arkush et al. 2002) and across neutral loci (Coltman et al. 1999). HFCs may be mediated through dominance (reduced expression of deleterious recessive alleles), overdominance (a fitness advantage based on having a wider variety of gene products; Tregenza and Wedell 2000), or through associative overdominance (heterozygotes at neutral loci have higher fitness than homozygotes due to an association with coding loci; Szulkin et al. 2010). Probably the best known examples of heterozygote advantage involve the major histocompatibility complex (MHC), which is involved in vertebrate immune function. Homozygosity at MHC has been implicated in spontaneous abortion in humans (Ober et al. 1998) and other primates (Knapp et al. 1996), whereas heterozygosity at MHC is associated with increased resistance to disease (e.g. Arkush et al. 2002). Studies investigating heterozygosity at noncoding loci such as microsatellites have generally assumed that multilocus heterozygosity accurately reflects genome-wide heterozygosity, or the degree to which an individual is outbred (eg Coltman et al. 1999). However, correlations between marker heterozygosity and fitness may instead reflect local effects whereby one or more of the loci under study are closely linked to coding loci that experience heterozygote advantage or disadvantage (Hansson and Westerberg 2002; Lieutenant-Gosselin and Bernatchez 2006). Such local effects may differ from global or genome-wide patterns in terms of the strength and even the direction of the relationship between heterozygosity and fitness (Lieutenant-Gosselin and Bernatchez 2006).

The magnitude and direction of the relationship between individual genetic diversity and fitness can also vary considerably depending on population structure and history. Outbreeding depression (a negative relationship between genetic diversity and fitness) can be caused by breaking up successful coevolved gene complexes, or by disrupting adaptations to the local environment (Neff 2004). For example, in a wild population of bluegill sunfish (*Lepomis macrochirus*), reproductive success was highest at an intermediate level of parental genomic divergence, presumably optimizing the balance between inbreeding and outbreeding depression (Neff 2004). Similarly, another study found both positive and negative local HFCs for a variety of fitness-related traits in threespine stickleback (Lieutenant-Gosselin and Bernatchez 2006).

In this study, I investigate how individual genetic diversity is related to fitness and parental care in song sparrows. Strong evidence has been reported for inbreeding depression in this species, based on pedigree analysis of a west coast insular population (Mandarte Island, British Columbia). For example, both cell-mediated immunity and male song repertoire size decline with inbreeding (Reid et al. 2005, 2007). Moreover, relatively outbred individuals in this island population were more likely to survive a population crash (Keller et al. 1994). Matings between relatives do occur, but produce fewer offspring, and such offspring have low survivorship (Keller 1998). However, the relationship between individual genetic diversity and fitness in non-insular song sparrows is much less well understood.

I examined the relationship between individual genetic diversity and fitness, and individual genetic diversity and parental care, in an Ontario population of song sparrows, *Melospiza melodia melodia*. Unlike the population studied by Reid and colleagues, song sparrows at our study site are non-insular, migratory, and because much of the surrounding landscape also contains song sparrows, presumably outbred. The study population has been monitored by our research group since 2002. To date, evidence for heterozygote advantage in this population has been mixed. In terms of reproductive success, Pfaff et al. (2007) found that genetic diversity is weakly correlated with song repertoire size, although it should be noted that the study used only a small number of loci (3) to assess genetic diversity. Because females of this species prefer large song repertoires (Searcy 1984), a positive relationship between genetic diversity and song complexity may provide heterozygous males with a mating advantage. Moreover, genetic similarity between mates was negatively related to nestling growth rate (Potvin and MacDougall-Shackleton 2009). This finding may reflect nestling heterozygote advantage and/or the fact that females mated to genetically dissimilar males provided more parental care to offspring (Potvin and MacDougall-Shackleton 2009). Among adult males infected with blood-borne parasites, severity of infection was negatively related to microsatellite heterozygosity (Singh 2007) suggesting a survivorship advantage for heterozygous individuals. However, in contrast to patterns observed on Mandarte Island, preliminary analysis has revealed no relationship between genetic diversity and cell-mediated immunity (skin swelling in response to phytohaemmaglutinin, PHA; E. MacDougall-Shackleton and J. Kewin, unpublished data). This may indicate that our outbred, migratory population does not experience severe inbreeding depression with respect to cell-mediated immunity, or may be due to the limited number of loci (7) used in the initial analysis. Thus, the benefits of individual genetic diversity and its influence (if any) on parental investment remain largely elusive.

My objective in this chapter is to determine whether individual genetic diversity influences survivorship and reproductive success. I quantified genetic diversity using 17 microsatellite loci and related this measure to a variety of measures of fitness. If HFCs provide strong advantages in terms of survival, I expect genetically diverse individuals to have greater immunocompetence (as measured by a bactericidal assay). Similarly, genetically diverse sparrows should be more likely than their less diverse counterparts to return to the breeding grounds the following year. Adult philopatry is very high in this population and this species, and individuals that breed at the study site in one year but do not return the following year are presumed dead (MacDougall-Shackleton et al. 2009). I also predict that genetically diverse nestlings should grow faster than their homozygous counterparts. Finally, if genetic diversity enhances survivorship, heterozygosity may be higher among breeding adults (who have already successfully completed development and survived to at least one year of age) than among newly hatched nestlings (who have not yet experienced as much potential selection against low genetic diversity).

If HFCs confer advantages to genetically diverse individuals in terms of reproductive success, I expect more heterozygous individuals to produce and fledge more offspring (as assessed by traditional counts as well as genetic analysis of parentage). Moreover, because heterozygote advantage is often related to sexually selected traits, I expect this pattern to be more pronounced in males than females. In addition, if genetically diverse individuals are better able to provide parental care, I expect genetically diverse adults to visit their nests more often. Finally, I investigate whether global or local effects are more likely to be driving any HFCs observed, by analyzing relationships locus by locus and based on various subsets of loci. Collectively, this study casts light on the extent to which individual genetic diversity is linked to fitness in an outbred population, and provides insight as to the processes maintaining genetic variation in natural populations.

2.2 Methods

Study population and site

Field work for this project was conducted between April 11th and June 24th, 2009. The study site supports about 30-40 pairs of song sparrows, *Melospiza melodia melodia*, breeding on the Bracken Tract of Queen's University Biological Station near Newboro, Ontario (44° 38' 60 N / 76° 19' 0 W). This site consists of approximately 1 square kilometre of forest edge and other open, old field habitat suitable for song sparrow breeding. Song sparrows in this part of Ontario are migratory, but show high adult philopatry: about half the adults breeding in one spring return the following spring, typically to the same territory (MacDougall-Shackleton et al. 2009). Predation on eggs and nestlings is the major cause of nest failure, with over half of all nests destroyed by predators in the 2007-2010 breeding seasons.

Field methods

I captured 24 adult female and 48 adult male song sparrows using seed-baited Potter traps during April and early May 2009. Each bird was banded with a unique combination of three colour bands for individual identification in the field, a Passive Integrated Transponder (PIT) tag (Biomark, Oregon; 12mm long x 2mm diameter) attached to a leg band for nest monitoring (described below), and a uniquely numbered Canadian Wildlife Service (CWS) band (CWS banding permit

#10691B). From each subject, I also collected a small blood sample (<50µL) from the brachial vein, of which part was blotted onto high wet strength filter paper for genetic analyses (described below). The remaining blood (about 25µL) was transferred to a cryogenic tube then snap-frozen in liquid nitrogen within 5 minutes of collection for subsequent bactericidal analysis (described below). Subjects were released at the site of capture.

Along with other members of our research group, I located nests based on behavioural observations, and monitored them until hatching time. On the second day after hatch (day 2), I collected a small (<15µL) blood sample from the tarsal vein of each nestling and blotted it onto filter paper for genetic analysis. I also weighed each nestling to the nearest 0.1g using a spring scale, and marked its tarsus with a felt-tip marker (combinations of left, right, front, back) so that I could later identify individuals within each nest. Eggs that had not hatched by this date were considered inviable and were collected for genetic analysis (Environment Canada collecting permit CA 0244). Four days later (day 6), I returned to the nest and again weighed each surviving nestling to the nearest 0.5g. I then calculated each nestling's growth rate as day 6 mass – day 2 mass divided by 4 days, for grams per day. Also on day 6, I collected a small blood sample (<15µL) from each nestling for bactericidal activity analyses, and snap froze it in liquid nitrogen as described above.

For each nest at which one or both parents had been outfitted with a PIT transponder, I recorded parental nest visits on days 2 and 6 after hatching. I placed an antenna wrapped in camouflage tape at the base of the nest and placed the reader (also wrapped in camouflage tape) as unobtrusively as possible, approximately 2 meters from the nest. Previous work by our research group has determined that the presence of a PIT antenna and reader does not affect nest success (Potvin and MacDougall-Shackleton 2009). I set the antennae and readers up between 0500 and 0600h and recorded all nest visits for the next 6-8 hours (depending on battery life). The reader recorded each time a PIT tagged adult came within 10cm of the antenna, and scanned for PIT tags every 10 seconds if the bird was still in the vicinity. Following Potvin and MacDougall-Shackleton (2009), I then calculated visits per hour per nestling for each parent. Because females in this species also spend substantial amounts of time brooding nestlings before the nestlings can thermoregulate, I also calculated the proportion of time females spent at the nest per hour.

To monitor nest success, I returned to the nest at day 12-14 after nest hatching (that is, two to four days after the expected fledging date) and used behavioural observations (one or both parents scolding human intruders or carrying food; fledgling begging sounds) to determine whether any nestlings had fledged.

Field and genetic data from 2007 and 2008 (used in overwinter return analyses) were collected by members of Dr. Beth MacDougall-Shackleton's research group (Potvin 2008). Members of our current research group recorded whether or not birds sampled in 2009 returned in April and May 2010.

Genetic methods

Genomic DNA was extracted from blood blotted onto filter paper using an ammonium-acetate based extraction protocol modified from Laitinen et al. (1994). I then measured DNA concentration with a spectrophotometer, and diluted as necessary with sterile water to a final working concentration.

All individuals in the population (males, females and nestlings) were genotyped at eighteen microsatellite loci: Mme 1, Mme 2, Mme 7 (sex-linked) and Mme 12 (Jeffrey et al 2001); Escμ1 (Hanotte et al 1994); Pdoμ5 (Griffith et al 1999); SOSP 1, SOSP 2, SOSP3, SOSP 4, SOSP 5, SOSP 7, SOSP 9, SOSP 13, and SOSP 14 (Dr. Lukas Keller, pers. comm. to Dr. Beth MacDougall-Shackleton); and Zole B03, Zole C02, and Zole H05 (Poesel et al. 2008). One primer at each locus was dye-labelled (Integrated DNA Technologies or Applied Biosystems) and microsatellites were amplified using the polymerase chain reaction (PCR).

Each PCR reaction included 10mM Tris-HCl, 50mM KCl, 0.1% Triton X-100, 0.2mg/mL BSA, 2.5mM $MgCl_2$, 0.2mM of each dNTP, 0.1-0.4mM of each primer, 0.5U *Taq* polymerase (Fisher Scientific) and approximately 25ng of genomic DNA, in a total volume of 10μL. Cycling conditions included an initial step of either 180s at 94°C (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, H05, Mme 1, 12) or 270s at 94°C (Mme 2, 7, Escμ1, Pdoμ5), followed by 28 cycles of either 30 s at 94°C, 90s at the annealing temperature, and 60s at 72°C (SOSP 1, 2, 3, 4, 5, 7, 9, 13, 14, Zole B03, C02, H05, Mme 1, 12) or of 30s at 94°C, 40 s at the annealing temperature, and 40s at 72°C (Mme 2, 7, Escµ1, Pdoµ5). All reactions had a final step of 270s at 72°C. Annealing temperatures were 57°C for SOSP 2, 3, 4, 9, 13, 14, Mme 1, 12, Zole B03, C02, H05, 55°C for SOSP 1, 5, 7 and dropped from 52°C to 48°C using a touchdown reaction for Mme 2, 7, Escµ1 and Pdoµ5. PCR products were then sized on an Applied Biosystems 3130 Genetic Analyzer, following the manufacturer's protocol, and scored manually using Gene Mapper.

One locus, Zole H05, was fixed in our population with a single allele of 248bp observed. Because this locus was thus uninformative for either parentage or genetic diversity I have excluded it entirely from all subsequent analyses. All analyses reported below used the remaining 17 loci unless otherwise specified.

I tested all loci for deviations from Hardy-Weinberg equilibrium and for the possibility of null (nonamplifying) alleles, using a variety of approaches. First, I used IR Macro N4 (Amos et al. 2001) to estimate null allele frequencies based on observed and expected homozygote frequencies. Using a 3 year (2007-2009, n = 235 adults) dataset of unique and presumably unrelated individuals I found expected frequencies of null alleles greater than 0.1 at four loci: Mme 2 (0.112), Mme 12 (0.126), Sosp 5 (0.159), and Zole B03 (0.232). Using Cervus 3.0 (Marshall 1998) I found that these deviations from Hardy-Weinberg equilibrium were significant even after Bonferroni corrections, and moreover that a fifth locus (Sosp 7) was also predicted to harbour null alleles. I also used the Brookfield estimator of null allele frequency (1996) implemented in the program Micro-Checker (Oosterhout et al. 2004); this method identified only Mme 2, Sosp 5 and

Zole B03 as having frequencies of null alleles higher than 0.1. However, in order to be conservative, I omitted all five questionable loci from parentage analysis. Similarly, I performed all heterozygosity analyses described below both with and without the four questionable loci that were predicted, by the IR macro N4 (described below) used in this study, to harbour null alleles. The results presented below were calculated using all 17 microsatellite loci, except for paternity tests, as described above, because all findings were qualitatively identical regardless of whether these four loci were included (data not shown). This suggests that potential null alleles were not driving any relationships found. Moreover, standardized heterozygosity (SH) as calculated with all seventeen loci was highly and significantly correlated to SH as calculated without the four suspect loci (Figure 2.1, $r_{73} = 0.820$, $p < 0.001$).

I also used Micro-Checker to identify other potential problems that may sometimes affect the scoring of microsatellite loci (Oosterhout et al. 2004). I found no evidence of scoring errors due to large allele dropout at any of the seventeen loci. The observed deviations from Hardy-Weinberg were generally due to homozygote excess at most allele classes, suggesting the presence of null alleles rather than selection favouring a specific homozygous genotype.

I assessed genetic diversity for all individuals (males, females and nestlings) using the Excel-based IR Macro N4 developed by William Amos (2001). Specifically, I calculated standardized heterozygosity (SH; Coltman et al. 1999) which is the ratio of an individual's heterozygosity at a given locus to the average expected heterozygosity at that locus as calculated by allele frequencies.

This measure gives more weight to heterozygosity at less variable loci (Coltman et al. 1999). There are several ways to quantify individual genetic diversity and all are highly correlated (Acevedo-Whitehouse et al. 2009) but multi-locus heterozygosity or SH have been found to outperform stepwise mutation models of genetic diversity (mean d^2) and provide a more robust measure of genetic diversity in most cases (Slate and Pemberton 2002; Hansson 2010). I selected SH because it is a measure of individual genetic diversity that is appropriate to use when loci are sex-linked and allows for variation among loci in terms of allelic variability (Amos et al. 2001). It also eliminates any bias of having some individuals untyped at specific loci, due to PCR failure (Slate et al. 2000). Nestling sex was not determined in the course of this project, and so the sex-linked locus Mme 7 was excluded for all nestlings. Females were coded as having missing data for their second allele at this locus.

Fitness measures

Bactericidal assay

To measure innate immunity (following a protocol from Dr. Dan Ardia), I made a working dilution daily of 1:1 *Escherichia coli* ATCC # 8739 (Microbiologics #0483E7) with Phosphate Buffered Saline (PBS) that yielded approximately 100-200 colonies of *E. coli*. 20µL of this dilution was mixed with 90µL of CO_2 independent cell medium buffer and 10µL of whole blood, and incubated at 37°C for 45 minutes. The suspension was then plated in duplicate on thin tryptic soy agarose Petri dishes, spread evenly, and inverted when dry. The plates were incubated overnight at 41°C. One negative control without *E. coli* or blood was plated daily to ensure that the plates and environment were free of contamination. An *E. coli* control (cell medium buffer and *E.* coli) was plated before and after every 10 avian replicates to show the growth of *E. coli* without blood.

I counted *E. coli* colonies on each plate and averaged the two replicates for each bird. I then determined the percentage of bacteria killed for each bird relative to the *E. coli* control. In cases where there was no bacterial killing and sample plates had more *E. coli* colonies than controls (N = 6 of 58 nestlings and 7 of 70 adults), killing ability was categorized as 0% (following Matson et al. 2006).

Parentage analysis

To assess parentage of nestlings, I compared the microsatellite profiles of nestlings to those of their social parents at six microsatellite loci (SOSP 13, SOSP 3, SOSP 14, Mme 2, Escμ1, Pdoμ5). All nestlings had genotypes consistent with those of their social mother. In cases of at least one mismatch between offspring and social father, an additional panel of microsatellite profiles was used (SOSP 9, SOSP 2, SOSP 4, Mme 1) to confirm exclusion. With known maternity, Cervus 3.0 predicted a >99.99% exclusion probability for this dataset (Kalinowski et al. 2007). In all cases, nestlings identified as extra-pair offspring mismatched their social father at 2 or more loci of the 10 loci screened. I attempted to identify the genetic fathers of any extra-pair offspring based on the microsatellite profiles of the other males in the population, but in most cases none of the males genotyped had microsatellite profiles consistent with those of extra-pair offspring, suggesting that the genetic sires of such offspring were either transient or held territories outside the study area.

Statistical analyses

All variables were tested for normality using the Kolmogorov-Smirnov test and visual inspection, and were found to be normally distributed. All analyses were done with SPSS (2008) or PASW (2009). All statistical tests were two-tailed. Following Nakagawa (2004), I have presented effect sizes alongside exact *p* values.

To study the relationship between genetic diversity and bacterial killing ability, I conducted linear regressions with SH as the independent variable and bactericidal ability as the dependent variable. I separated nestlings and adults for all analyses, and included the length of time blood had been kept frozen before bactericidal assay as a covariate and sex (in the case of adults) as a factor. However, preliminary analyses demonstrated that days kept in freezer, date sampled, and sex did not explain significant variation in killing ability (data not shown), and so the results reported below are based on simple linear regressions. To determine whether age or sex affects bacterial killing ability, I conducted a one-way ANOVA and Tukey's post-hoc test with age and sex categories (adult male, adult female, or nestling) as the independent variables and killing ability as the dependent variable.

To investigate the relationship between SH and overwinter survival I used logistic regressions to relate SH (independent variable) to apparent overwinter survival (based on whether or not an individual returned to the study site the following year). As noted above, adult philopatry is high in this species (Nice 1941) and in this population (MacDougall-Shackleton et al. 2009) and so birds that are not observed to have returned in the spring are assumed to have died overwinter (MacDougall-Shackleton et al. 2009). Return data were collected for winters 2007-2008, 2008-2009, and 2009-2010. This model included sex as a factor and was run separately for each year.

I used logistic regressions to investigate the relationship between SH and clutch size (females only), and the relationship between SH and the maintenance of full paternity within the nest (males only). Likewise, I conducted logistic regressions to examine the relationship between SH and fledging success (that is, whether or not a nest succeeded in producing at least one fledgling) for each sex.

I used linear regressions to analyze parental care with SH as the independent variable and visits/nestling/hour on each day and for each sex as the dependent variables. I first included Julian date, presence of extra-pair young, presence of cowbirds, and weather conditions (categorized as "fair" or "rainy") as independent variables, but none explained a significant proportion of variation in any of the models tested (data not presented). I then eliminated these non-significant predictors and presented the simplest model. Variation in sample sizes in the following analyses reflects the facts that not all nestlings survived to day 6 and that not all adults bred at the study site.

Local versus global effects

For any significant heterozygosity-fitness correlations observed, I conducted one by one locus dropouts (following Ortego et al. 2007) in order to determine whether one or more loci contributed disproportionately to the observed relationship as predicted by the local effects hypothesis.

To determine whether SH was likely to reflect genome wide diversity, SH was calculated at two randomly assigned subsets of loci (N = 8, 9) using a jackknife approach (Excel Macro written in Visual Basic by Dr. Shawn Garner) and Pearson's correlation was calculated to determine the degree to which SH at one subset was related to SH at the other subset. This process was repeated a total of 1000 times to determine a distribution of correlation coefficients (*r*).

2.3 Results

Survivorship:

Bacterial killing ability

SH did not predict bacterial killing ability for adults (Figure 2.2a. $\beta_{66} = 6.987 \pm 19.052$, $r^2 = 0.002$, $p = 0.719$) or for nestlings (Figure 2.2b., $\beta_{42} = -21.168 \pm 26.180$, $r^2 = 0.017$, $p = 0.411$). However, bacterial killing ability did vary significantly based on age and sex (Figure 2.3., ANOVA, $F_{126} = 3.234$, $p = 0.033$). Specifically, adult females had significantly greater bactericidal ability than nestlings; adult males had intermediate killing ability.

Overwinter survival

Among adult song sparrows captured at the study site in 2008, SH was significantly and negatively related to the probability of returning in 2009 (Figure 2.4; $\beta_{108} = -5.440 \pm 1.665$, Wald = 10.680, $p = 0.001$). That is, genetically diverse individuals were less likely to return to the breeding site the following spring. This model also included sex as a categorical factor and showed that males were more likely than females to return in 2009 ($\beta_{108} = -1.536 \pm 0.507$, Wald = 9.175, $p = 0.002$). When only males were tested, the same relationship was found between SH and overwinter return (Figure 2.5a., $\beta_{58} = -7.160 \pm 2.512$, Wald = 8.122, $p = 0.004$). However, in females this relationship only approached, but did not reach, statistical significance (Figure 2.5b., $\beta_{50} = -3.820 \pm 2.209$, Wald = 2.989, $p = 0.084$).

To investigate the robustness of the observed relationship between SH and apparent survivorship, I randomly resampled 33 individuals (the number of birds sampled in 2008 that returned in 2009) from the 2008 dataset, and calculated the average SH of that subset. The average SH of these random samples ($n = 1000$) was significantly higher than the observed SH of returning birds in 2009 (Figure 2.6, $p = 0.004$).

Among birds sampled in 2009, the negative relationship between SH and returning to the breeding site in 2010 approached, but did not reach statistical significance (Figure 2.7, $\beta_{72} = -2.751 \pm 1.601$, Wald = 2.952, $p = 0.086$). Again, males were significantly more likely than females to return ($\beta_{72} = -1.517 \pm 0.611$, Wald = 6.175, $p = 0.013$). Genetically diverse males were significantly less likely to return (Figure 2.8a, $\beta_{47} = -4.991 \pm 2.216$, Wald = 5.073, $p = 0.024$) but there was no relationship between SH and probability of returning for females (Figure 2.8b, $\beta_{24} = 0.942 \pm 2.863$, Wald = 0.108, $p = 0.742$). Randomly resampling the 2009 dataset (as above) demonstrated that the mean SH of the subset of birds that returned to the breeding site in 2010 were not significantly less diverse than random expectations (Figure 2.9., $p = 0.239$).

Unlike other years investigated, I observed no relationship between SH and return rates from 2007 to 2008 (Figure 2.10., $\beta_{105} = 0.033 \pm 1.859$, Wald = 0.0003, $p = 0.986$). Neither did sex significantly predict the likelihood of return, although I did observe a trend that males were more likely than females to return in 2008 ($\beta_{105} = -0.796 \pm 0.449$, Wald = 3.137, $p = 0.077$). SH did not predict likelihood of return for males (Figure 2.11a., $\beta_{56} = -1.469 \pm 2.247$, Wald = 0.428, $p = 0.513$) or females (Figure 2.11b., $\beta_{47} = 3.568 \pm 3.473$, Wald = 1.056, $p = 0.304$) when analyzed separately.

The average SH of adults was significantly lower than that of nestlings (0.8291 and 0.8766 respectively; Figure 2.12, Independent samples t-test, $t_{175} = 2.014$, $p = 0.046$).

Nestling growth rates

SH of nestlings did not predict their growth rates from day 2 to day 6 after hatching (Figure 2.13, $\beta_{47} = 1.654 \pm 1.245$, $r^2 = 0.037$, $p = 0.191$). A post-hoc power analysis (using G*Power 3.1, Faul et al. 2009) shows that we have 80%

power to detect an effect size of $r = 0.385$ or higher, and a 99.8% chance of detecting the effect size reported in this population previously (Potvin and MacDougall-Shackleton 2009).

Reproductive success:

SH did not predict fledging success for males (Figure 2.14a., $\beta_{24} = 5.078 \pm 3.639$, Wald = 1.948, $p = 0.163$) or for females (Figure 2.14b., $\beta_{20} = 3.604 \pm 3.450$, Wald = 1.091, $p = 0.296$). Female SH did not predict clutch size (Figure 2.15, $\beta_{20} = 5.538 \pm 3.883$, Wald = 2.034, $p = 0.154$) nor did male SH predict the likelihood maintaining full paternity within the social mate's offspring (Figure 2.16, $\beta_{23} = -5.416 \pm 4.427$, Wald = 1.497, $p = 0.221$).

Parental care

Male SH did not predict paternal visits per hour per nestling on day 2 (Figure 2.17a., $\beta_9 = 0.790 \pm 0.1039$, $r^2 = 0.039$, $p = 0.560$), but was positively related to paternal visits per hour per nestling on day 6 (Figure 2.17b, $\beta_7 = 4.209 \pm 0.923$, $r^2 = 0.768$, $p = 0.004$).

Female SH did not predict maternal nest visits per nestling per hour on day 2 (Figure 2.18a., $\beta_{13} = 3.680 \pm 4.949$, $r^2 = 0.001$, $p = 0.915$) or time spent at nest per hour (Figure 2.18b., $\beta_{13} = 1762.021 \pm 1301.809$, $r^2 = 0.034$, $p = 0.545$) on day 2. Similarly, female SH did not predict visits per nestling per hour (Figure 2.19a., $\beta_8 = 0.128 \pm 3.296$, $r^2 < 0.001$, $p = 0.970$) or time spent at the nest per hour (Figure 2.19b., $\beta_8 = -2842.331 \pm 2312.221$, $r^2 = 0.178$, $p = 0.259$) on day 6.

Local versus global effects in explaining observed HFCs:

To test whether local effects were driving the observed negative relationship between genetic diversity and overwinter survival, heterozygosity at each individual locus was analyzed relative to return, using a 2x2 contingency table for each locus. Among adults sampled in 2008, heterozygotes at SOSP 1 were significantly less likely than homozygotes at this locus to return to the breeding grounds in 2009. Among adults sampled in 2009, heterozygotes at SOSP 9 were significantly less likely than homozygotes to return to the breeding grounds in 2010. No other loci were significantly predictive of overwinter return rates in isolation (Table 2.1a, b).

To test whether the negative relationship between heterozygosity and overwinter return was driven primarily by genome wide (global) effects, I re-analyzed standardized heterozygosity with each locus dropped out one at a time following Ortego et al. (2007) and investigated this measure's ability to predict overwinter survival. Again, sex was included as a factor in the model. When analyzing 2008 birds, the significant negative relationship found between SH and returning to the breeding grounds in 2009 was lost when either SOSP 1 or SOSP 7 were omitted, and became a statistically non-significant trend ($0.05 < p > 0.1$) when Mme 1, Mme 2, Escμ1, Pdoμ5 or SOSP 5 were omitted (Table 2.2a). In 2009 birds when Mme 7, SOSP 9 and SOSP 13 were omitted the relationship between SH and return in 2010 was lost (Table 2.2b). In all analyses, sex was a significant predictor with males more likely to return and where significant relationships between SH and return were found, the trend was negative (data not shown).

On average, the SH-SH correlations obtained were not significantly different from zero (One sample t-test, $t_{999} = -0.049$, $p = 0.961$).

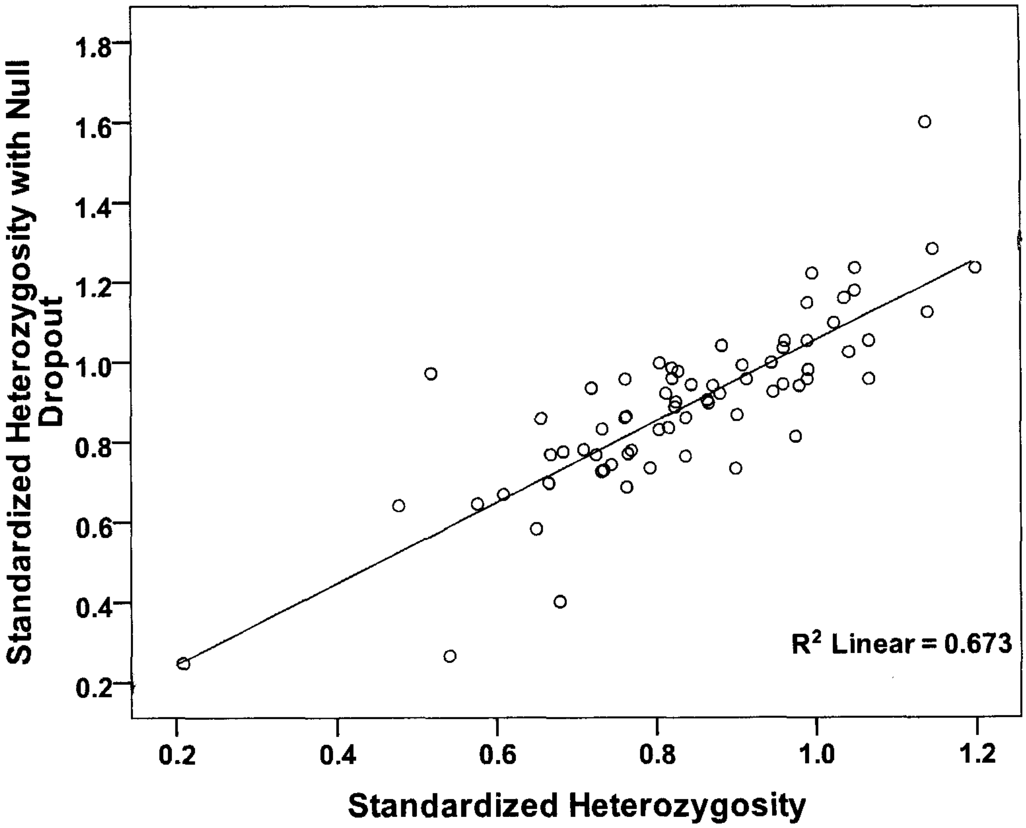

Figure 2.1. Standardized heterozygosity (SH) calculated including (abscissa) and excluding (ordinate) the 4 loci with suspected null alleles in song sparrows sampled in 2009 (N = 73).

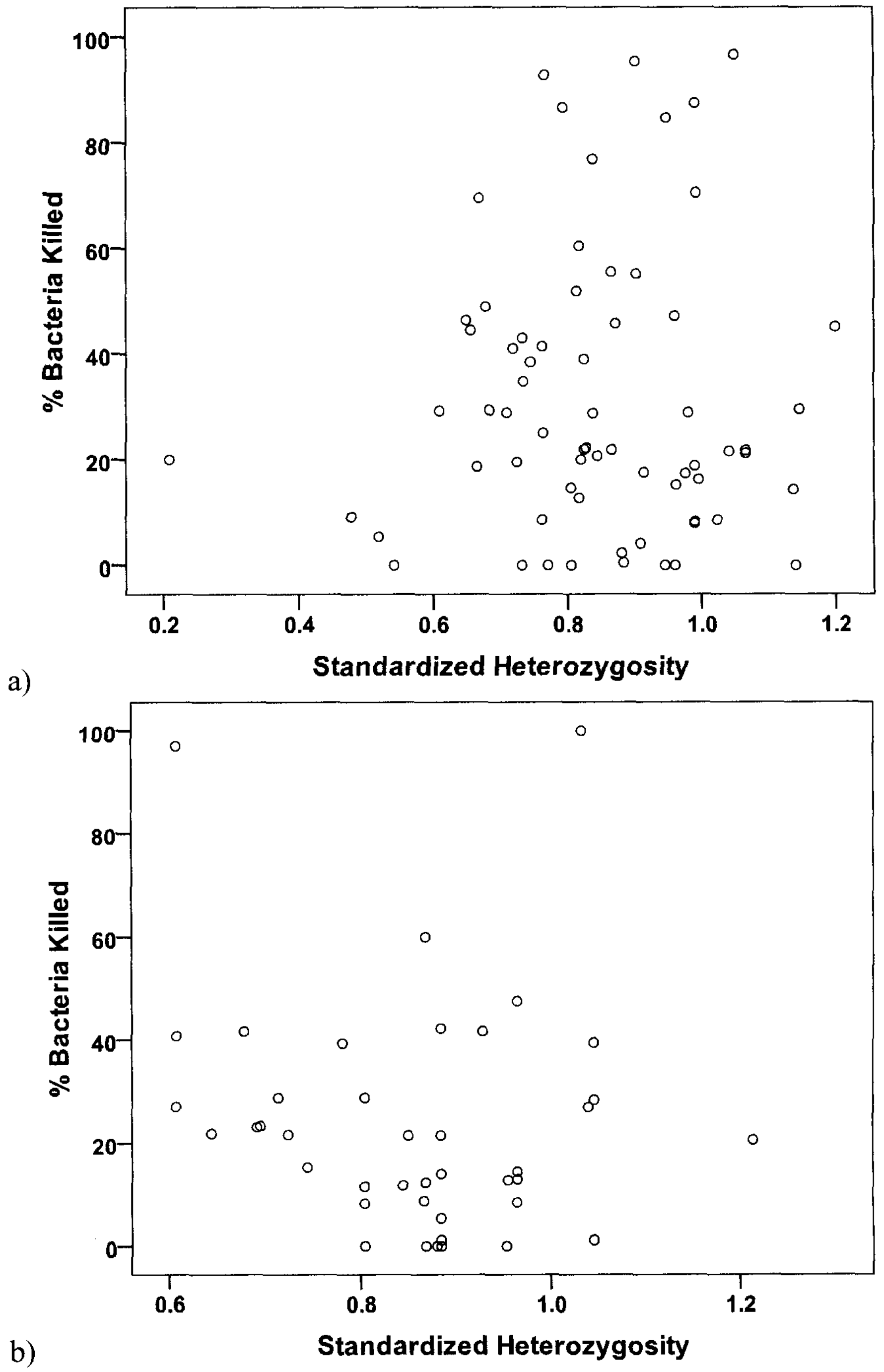

Figure 2.2. Percentage of *E. coli* killed as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. SH does not predict percent bactericidal ability of a) adults (N = 66) or b) day 6 after hatch nestlings (N = 42).

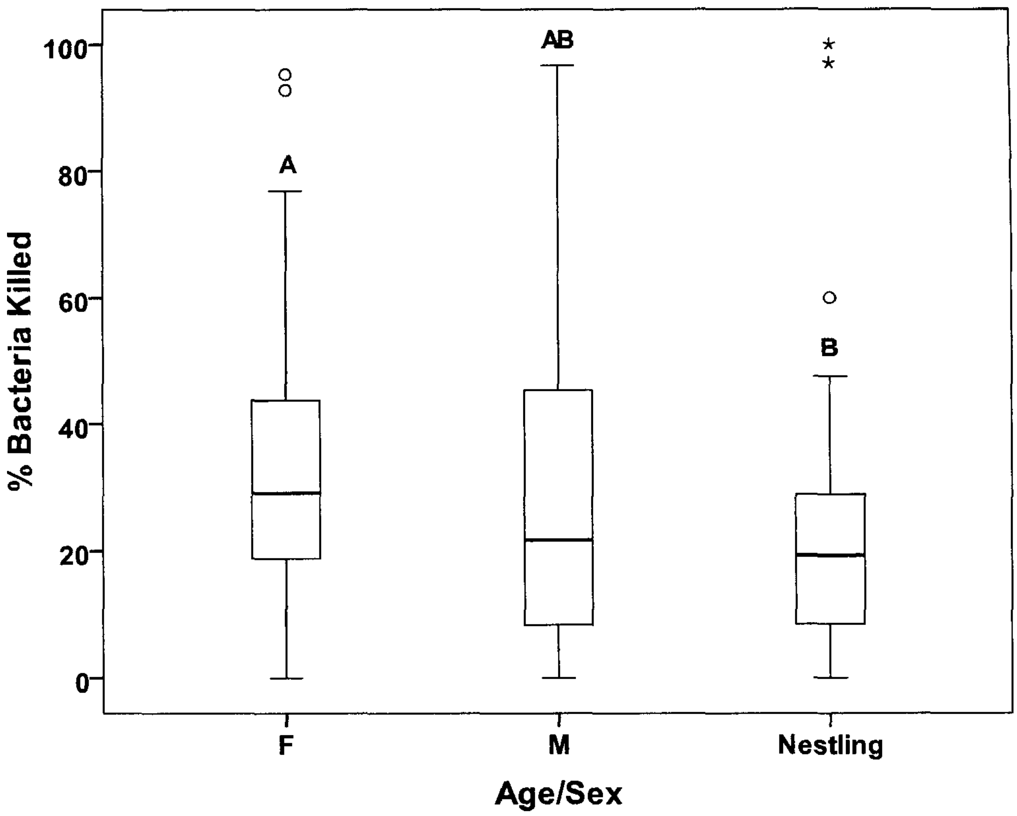

Figure 2.3. Percentage of *E. coli* killed as a function of age and sex categories in song sparrows sampled in 2009. Bacterial killing varies with age and sex. F = adult females (N = 22), M = adult males (N = 47), Nestling = six days of age, sex unknown (N = 58). Different letters (A, B) represent significant differences based on Tukey's post-hoc test. Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR. Asterisks represent points outside 3 x IQR.

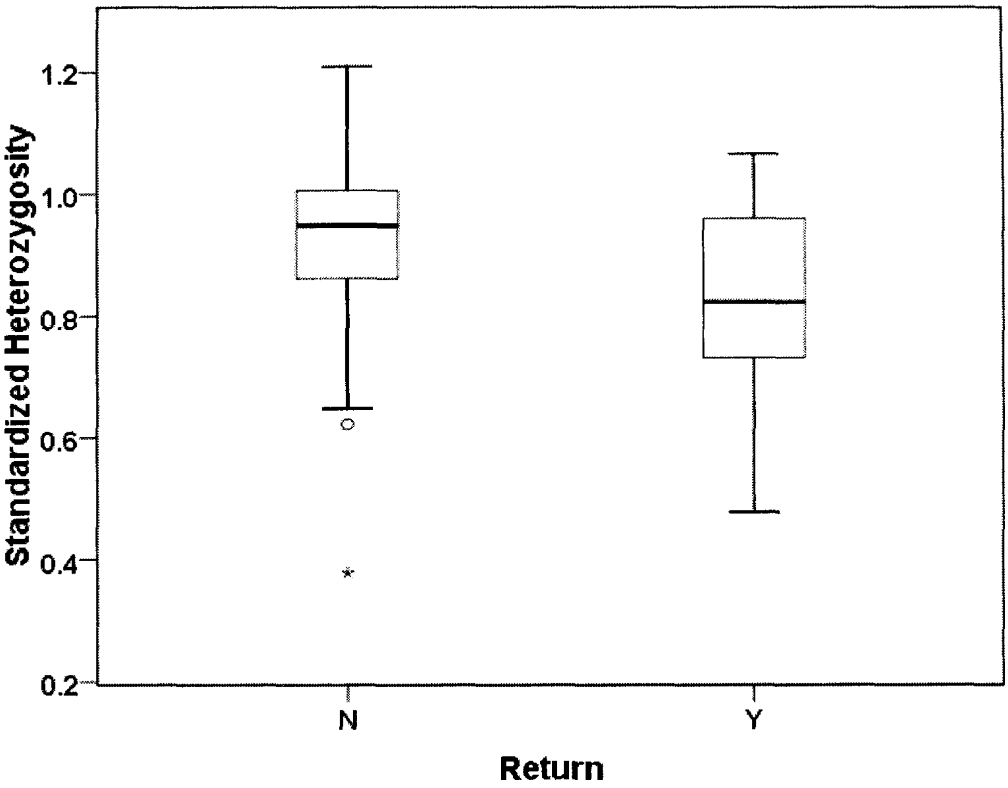

Figure 2.4. Overwinter return as a function of standardized heterozygosity (SH) in song sparrows sampled in 2008. SH of birds breeding in 2008 is negatively associated with the probability of returning in 2009 (N = 108). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR. Asterisks represent points outside 3 x IQR.

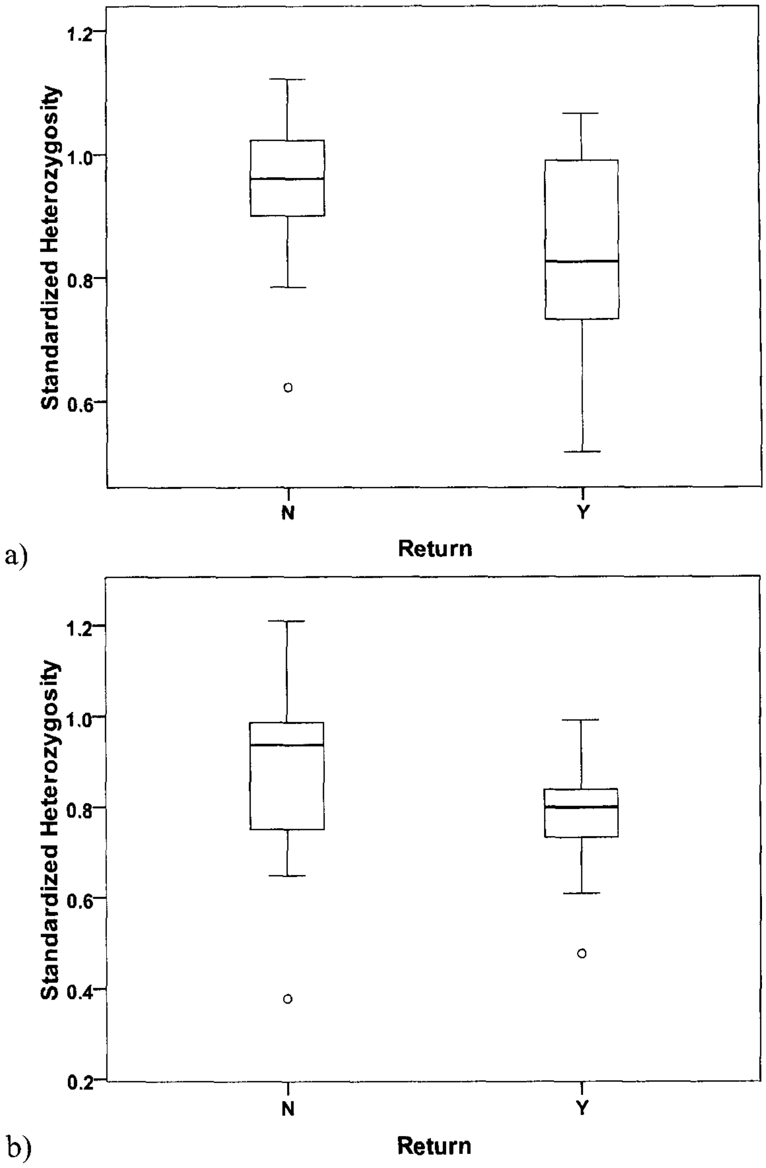

Figure 2.5. Overwinter return as a function of standardized heterozygosity (SH) in song sparrows sampled in 2008. SH in 2008 negatively predicts overwinter return in a) males (N = 58) but not b) females in 2009 (N = 50). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

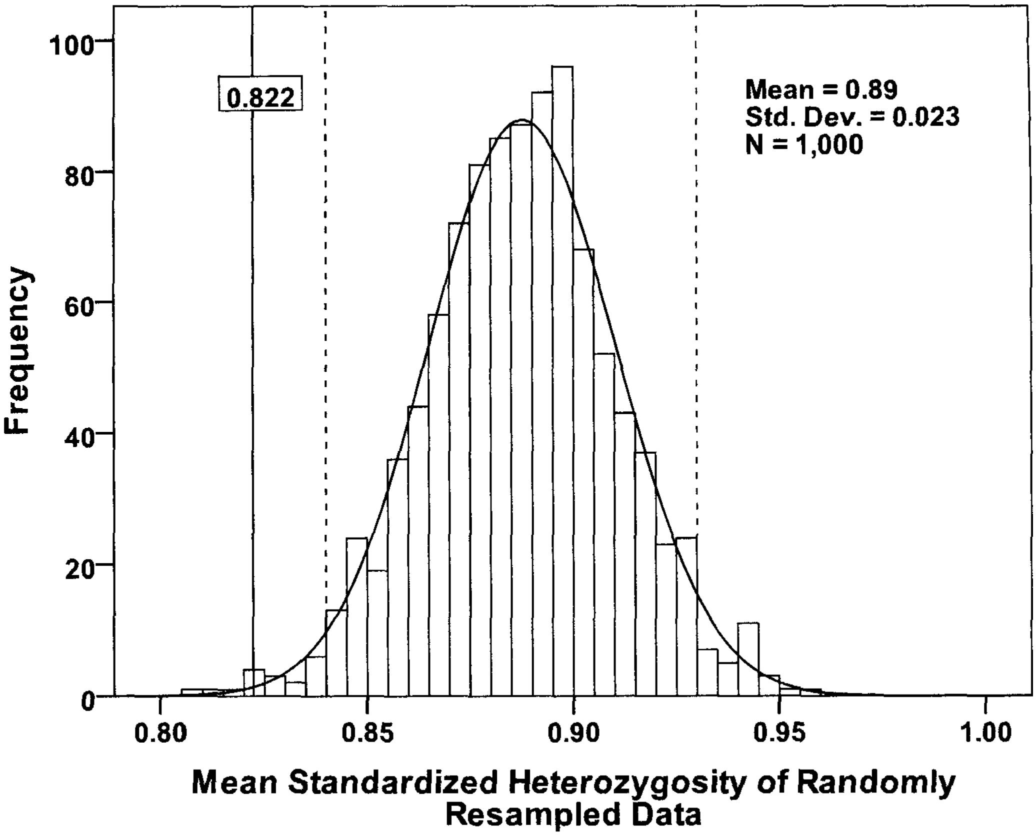

Figure 2.6. Random resamples of standardized heterozygosity (SH) of song sparrows sampled in 2008. Mean SH of birds that bred in 2008 and returned in 2009 (N = 33), as illustrated by the solid line, was significantly lower than random resamplings from the 2008 dataset (N = 1000). Dashed lines represent 95% confidence intervals of random resamplings, and the solid curve represents a normal distribution.

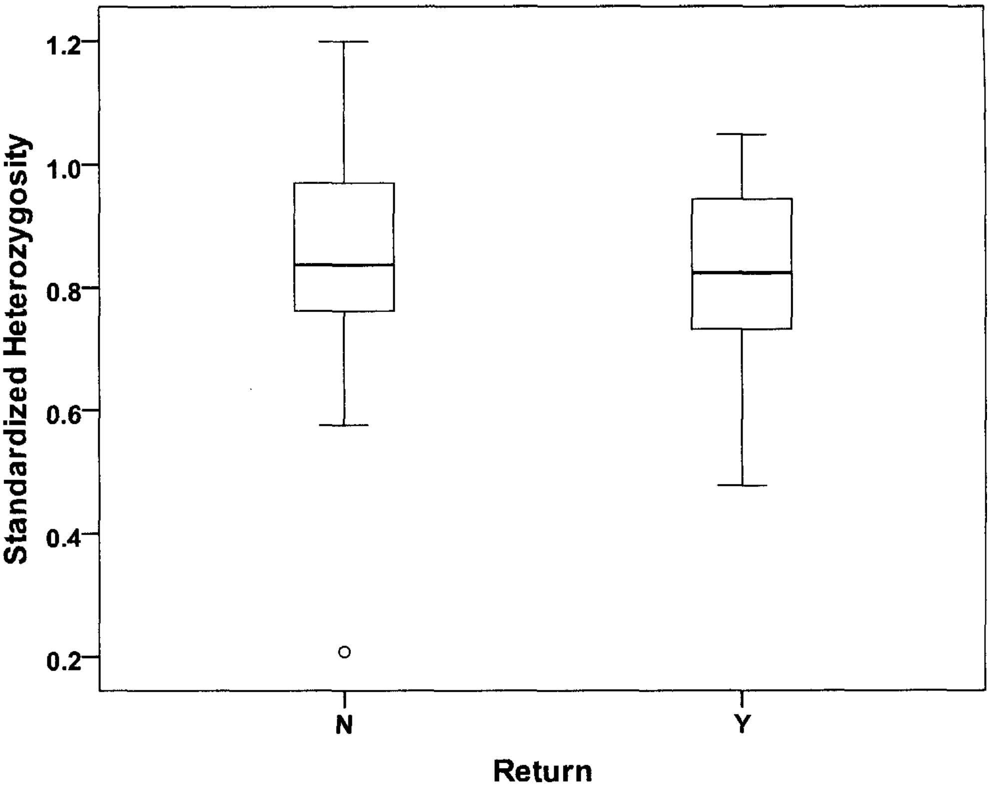

Figure 2.7. Overwinter return as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. SH of birds sampled in 2009 did not predict return in 2010 (N = 72). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

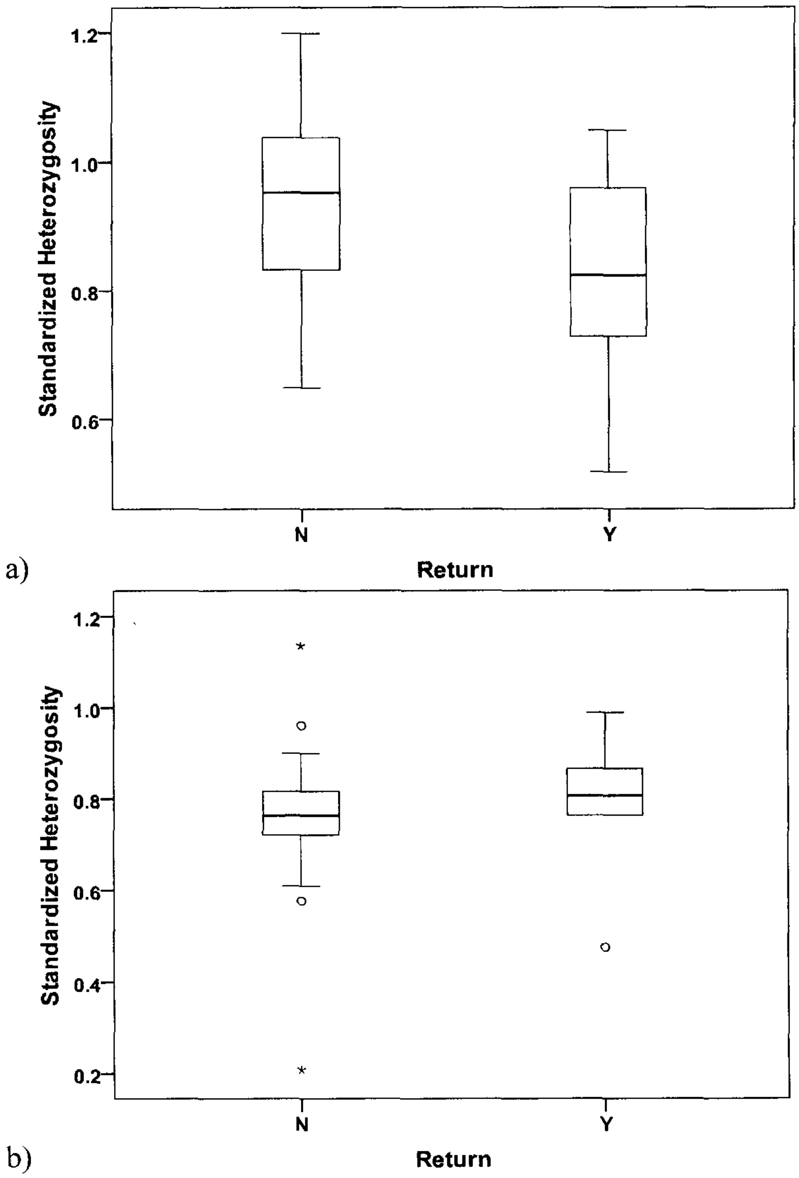

Figure 2.8. Overwinter return as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. SH predicts lower return in 2010 for a) males (N = 47) but not b) females (N = 24) sampled in 2009. Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR. Asterisks represent points outside 3 x IQR.

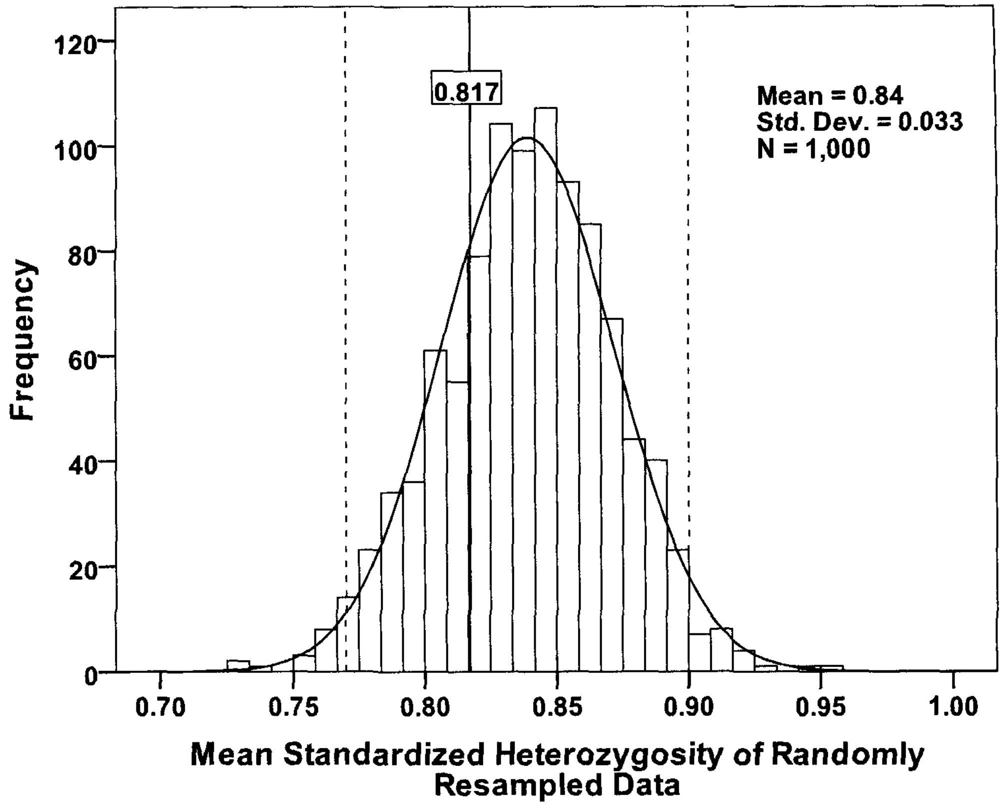

Figure 2.9. Random resamples of standardized heterozygosity (SH) of song sparrows sampled in 2009. Mean SH of birds that bred in 2008 and returned in 2009 (N = 30), as illustrated by the solid line, was significantly lower than random resamplings from the 2008 dataset (N = 1000). Dashed lines represent 95% confidence intervals of random resamplings, and the solid curve represents a normal distribution.

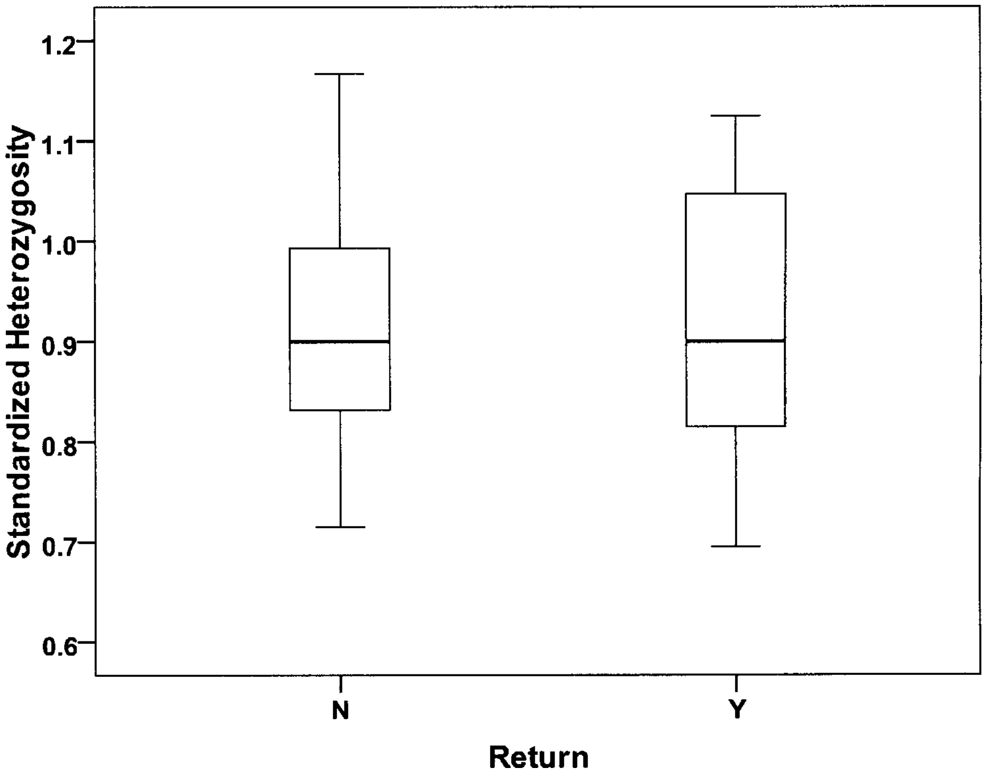

Figure 2.10. Overwinter return as a function of standardized heterozygosity (SH) of song sparrows sampled in 2007. SH of birds breeding in 2007 did not predict return in 2008 (N = 105). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent the maximum and minimum values.

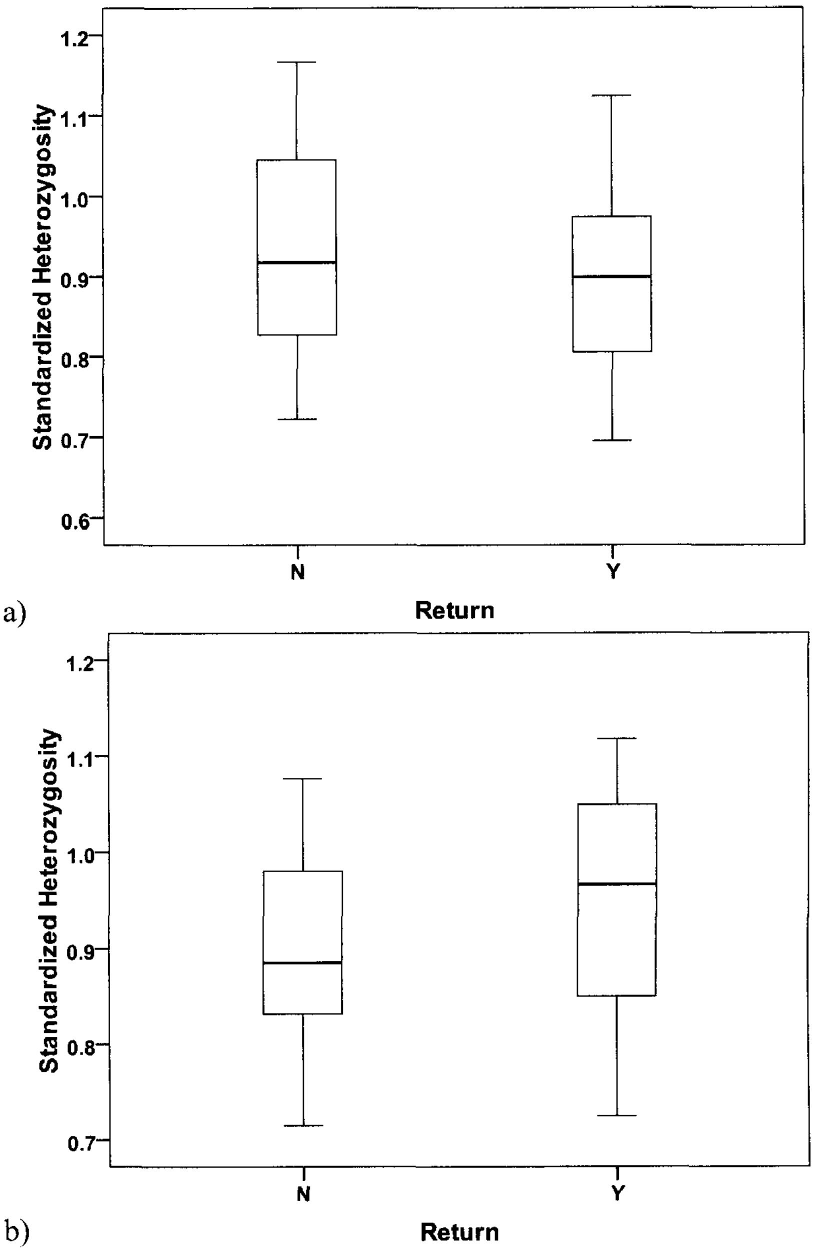

Figure 2.11. Overwinter return as a function of standardized heterozygosity (SH) of song sparrows sampled in 2007. SH of (a) males (N = 56) and (b) females (N = 47) sampled in 2007, as a predictor of overwinter return rates. SH was not related to the probability of return for either sex. Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent the maximum and minimum values.

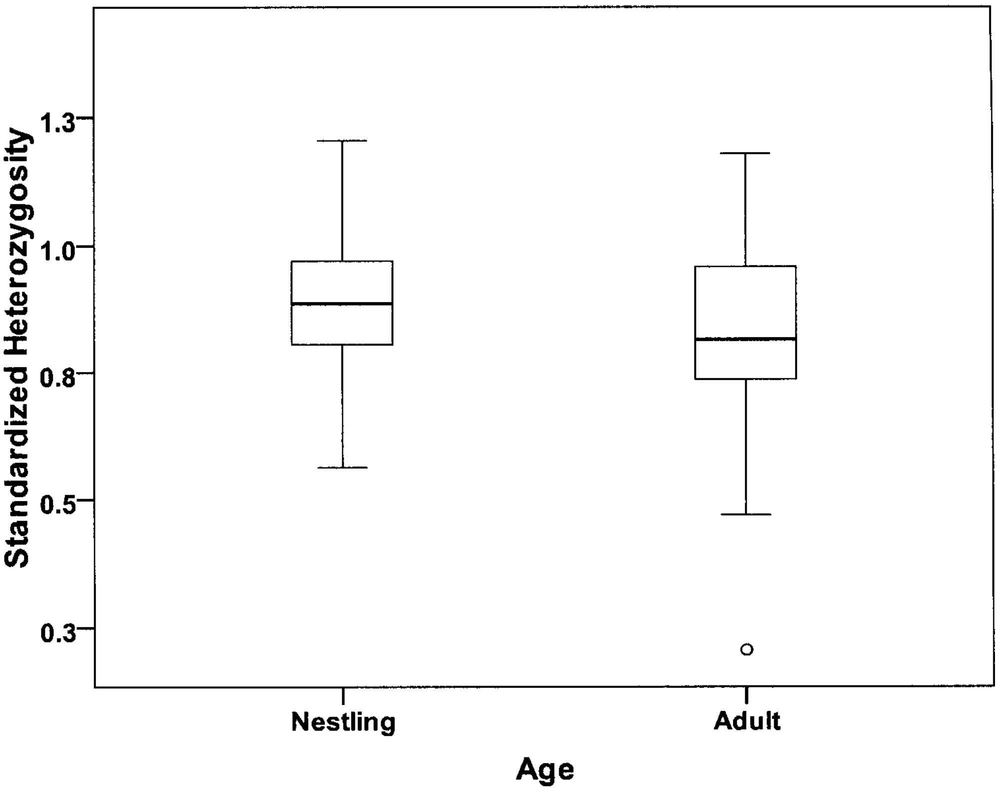

Figure 2.12. Age as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. SH of nestlings (N = 104) was significantly higher than that of adults (N = 73). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

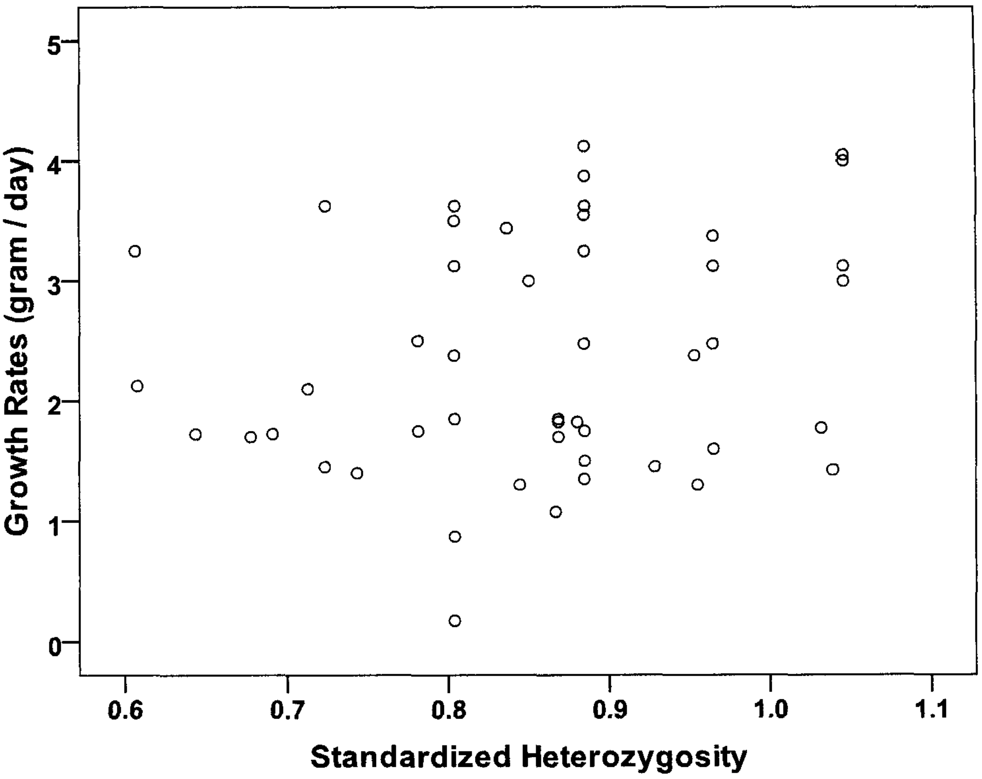

Figure 2.13. Nestling growth rate from day 2 to day 6 after hatch as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Nestling growth rates from day 2 to day 6 were not related to SH (N = 47).

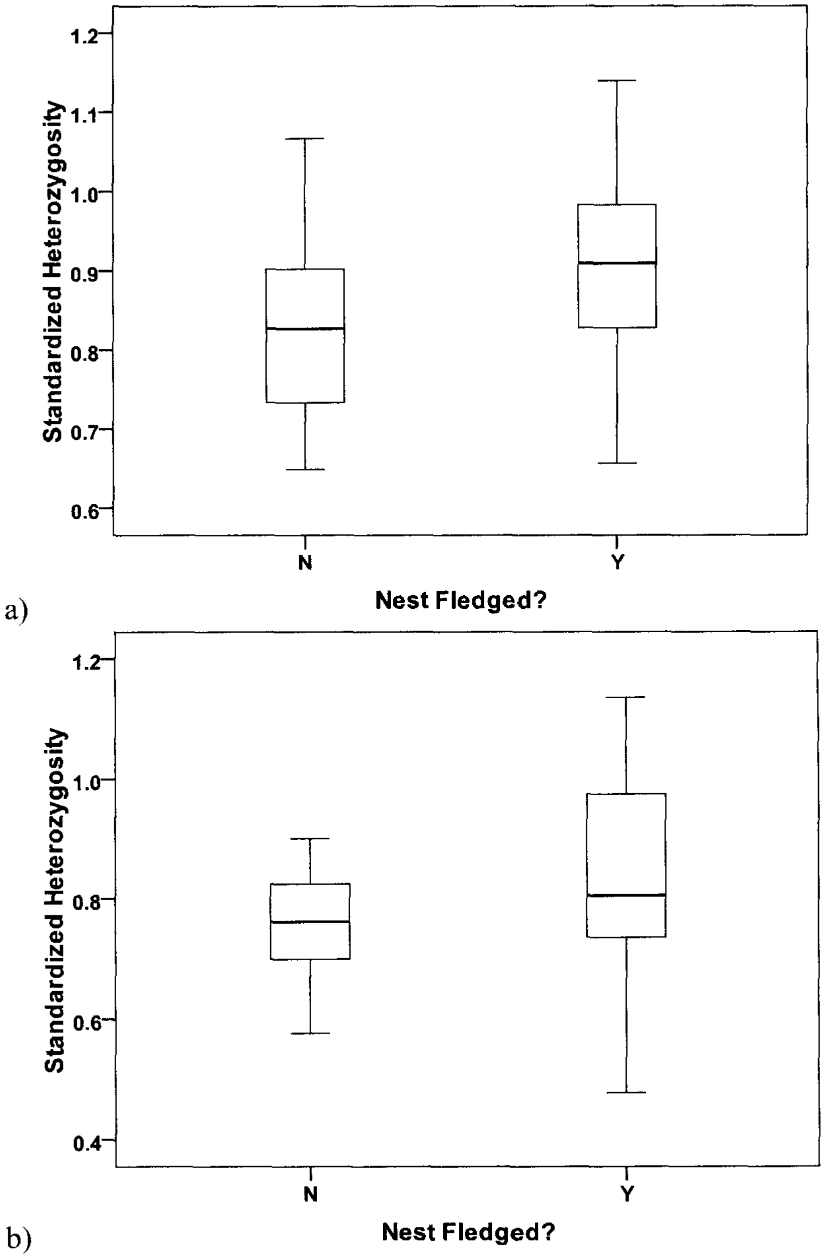

Figure 2.14. Fledging success as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. SH of parents does not predict fledging success for a) social fathers (N = 24) or b) mothers (N = 20). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent maximum and minimum values.

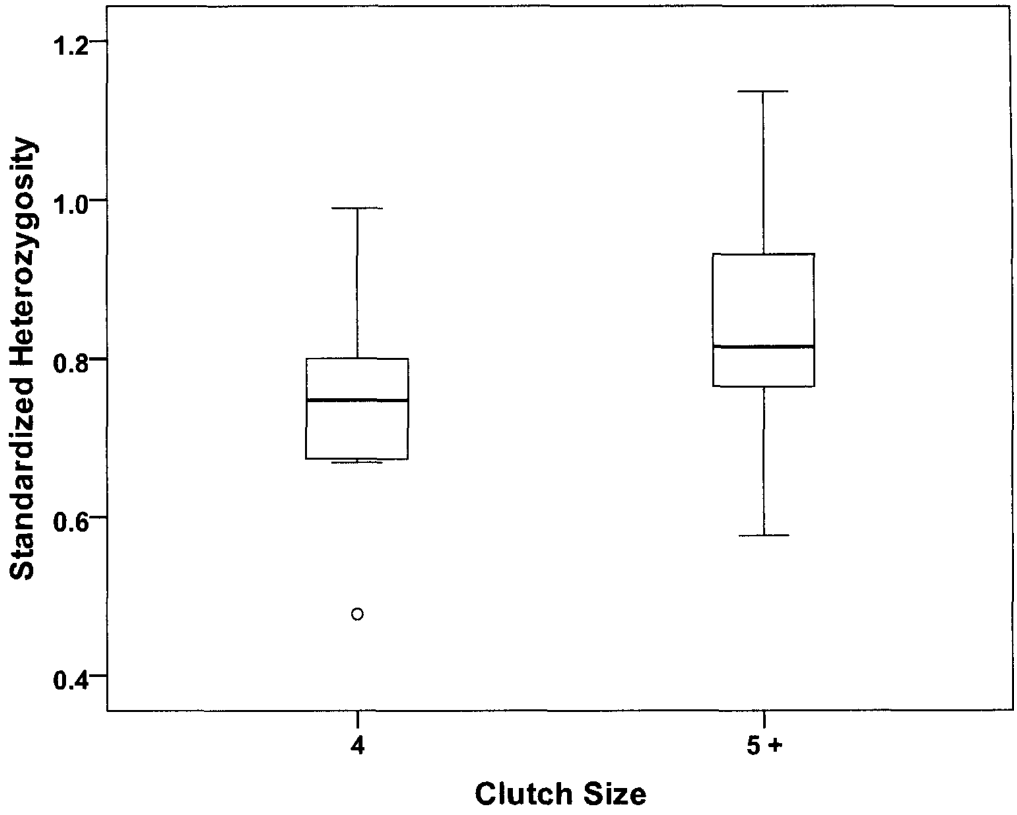

Figure 2.15. Clutch size as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Female SH did not predict clutch size. Boxes represent the 75th, 50th, and 25th percentiles for females with 4 eggs (N = 8) or 5 or more eggs (N_5 = 10, N_6 = 1). Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

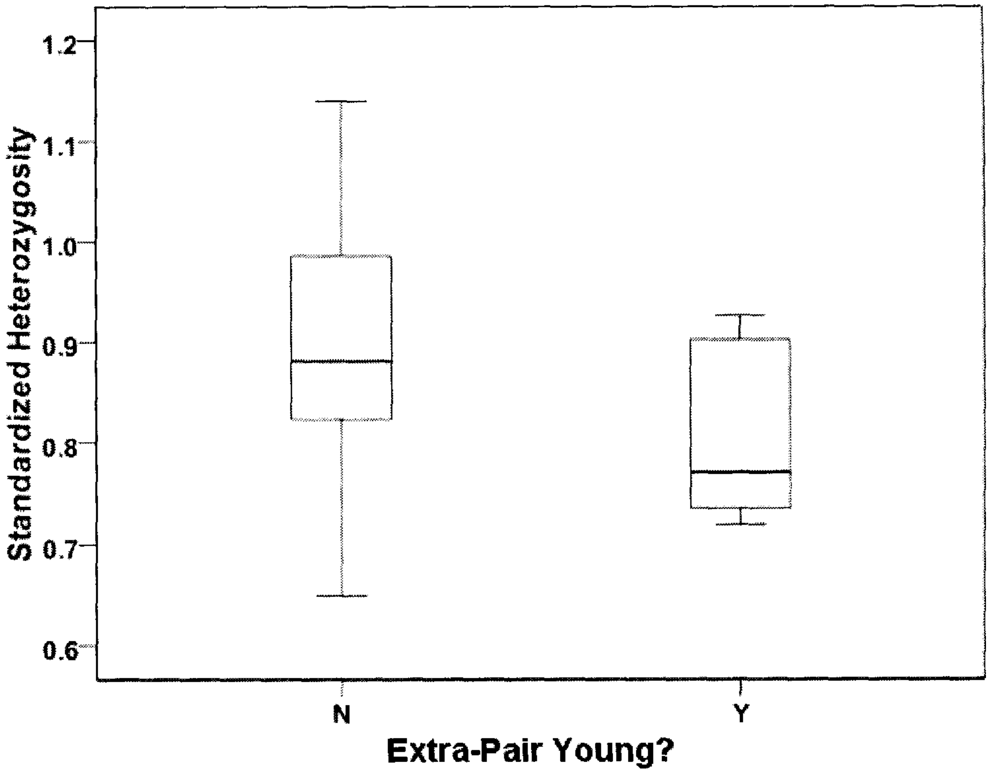

Figure 2.16. Presence of extra-pair young in nest as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Male SH did not predict the likelihood of losing paternity within social mate's offspring (N = 23). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent maximum and minimum values.

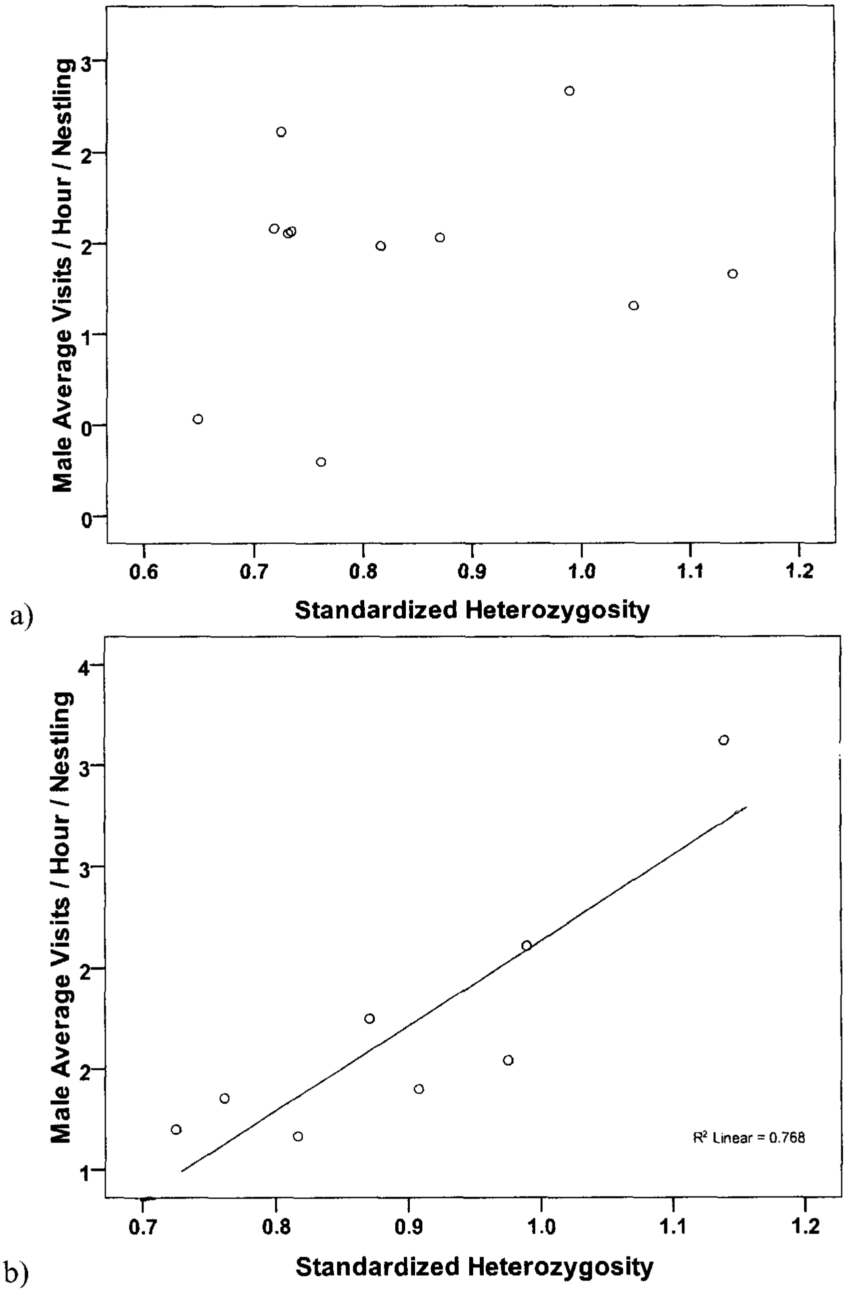

Figure 2.17. Male visitation rates as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Male SH did not predict male average visits per nestling per hour on a) day 2 (N = 11) but b) did predict visitation rate on day 6 (N = 8).

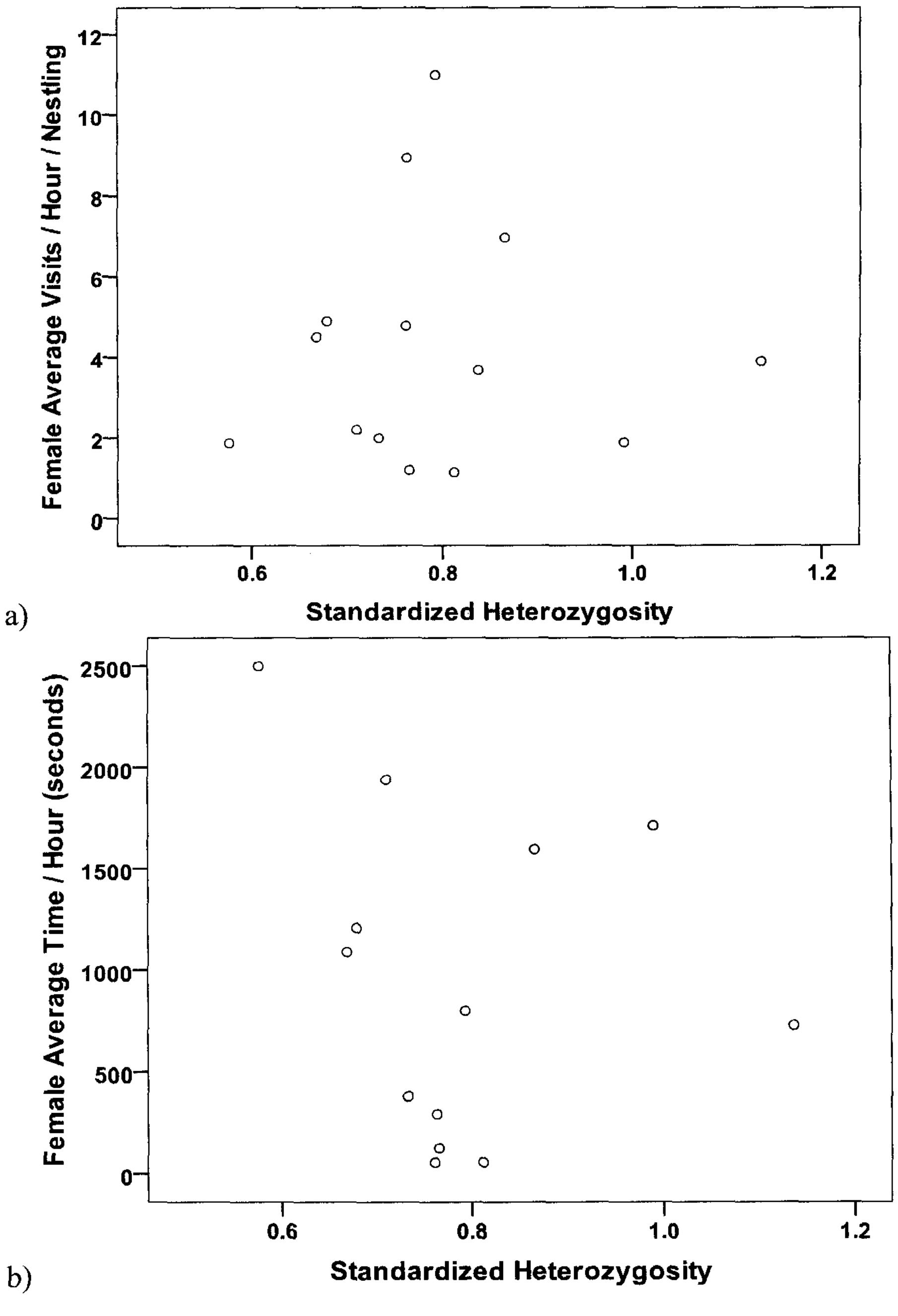

Figure 2.18. Female visitation time and rates as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Female SH did not predict a) visits per nestling per hour (N = 14) or b) time spent at nest per hour on day 2 (N = 14).

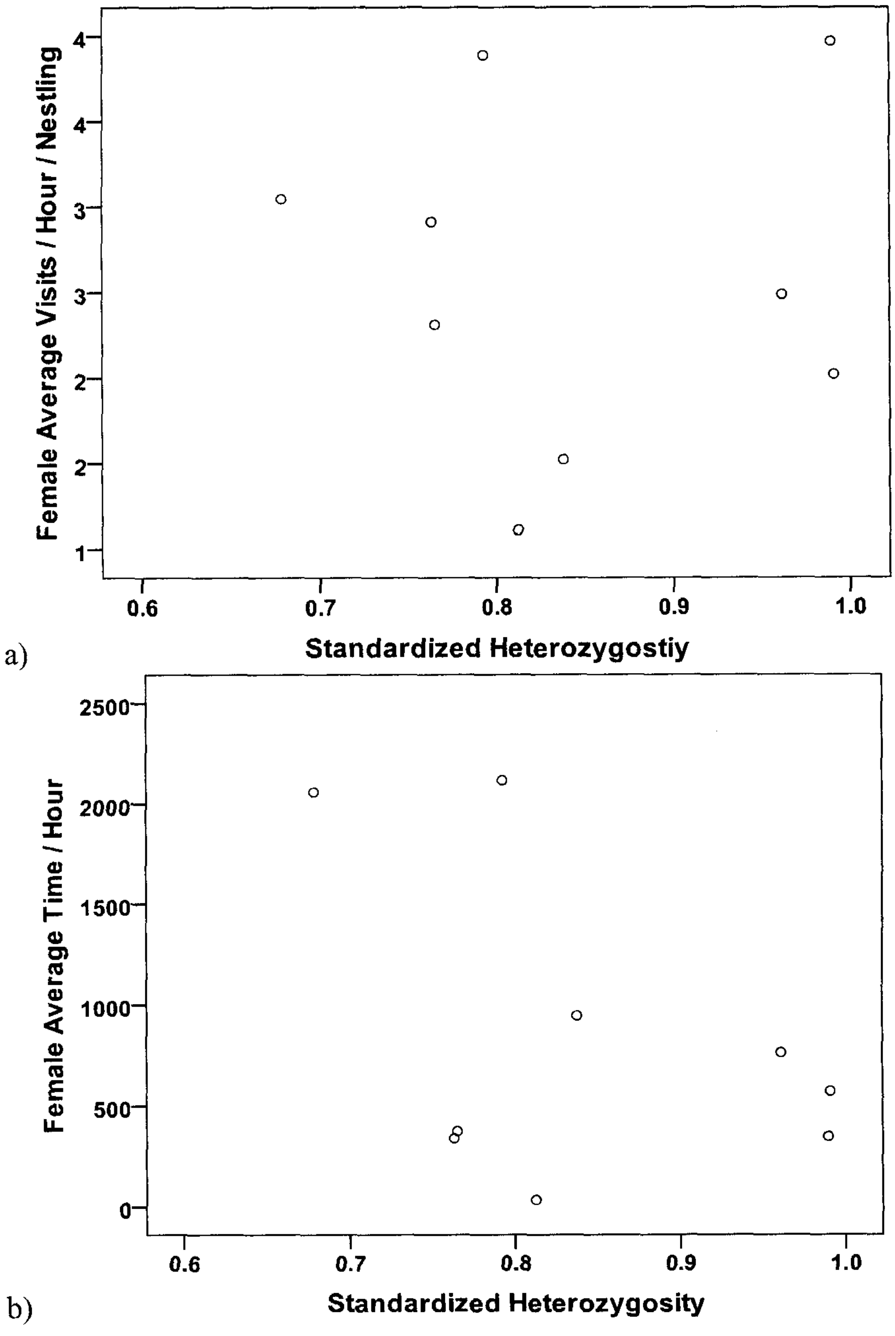

Figure 2.19. Female visitation time and rates as a function of standardized heterozygosity (SH) in song sparrows sampled in 2009. Female SH did not predict a) visits per nestling per hour (N = 9) or b) time per hour on day 6 (N = 9).

Table 2.1. Overwinter return as a function of single locus heterozygosity in song sparrows. Single-locus heterozygosity as a predictor of returning the following spring, for (a) birds sampled in 2008, (b) birds sampled in 2009. Asterisks indicate loci for which single-locus heterozygosity was negatively related to overwinter return in a given year.

a) **Locus Name**	χ^2	**P**
Mme 1	1.668	0.323
Mme 2	0.174	0.808
Mme 7	0.222	0.774
Mme 12	2.771	0.110
Escu 1	1.348	0.292
Pdou 5	1.277	0.430
SOSP 1 *	5.552	0.025 *
SOSP 2	0.382	0.643
SOSP 3	0.212	0.777
SOSP 4	0.012	1.000
SOSP 5	0.006	1.00
SOSP 7	1.032	0.401
SOSP 9	0.242	0.779
SOSP 13	0.457	0.582
SOSP 14	2.553	0.154
Zole B03	0.127	0.818
Zole C02	0.010	1.000

b) Locus Name	χ^2	**P**
Mme 1	0.384	0.535
Mme 2	0.243	0.622
Mme 7	1.444	0.229
Mme 12	1.012	0.315
Escu 1	2.329	0.127
Pdou 5	1.403	0.236
SOSP 1	0.120	0.729
SOSP 2	0.095	0.758
SOSP 3	1.556	0.212
SOSP 4	1.859	0.173
SOSP 5	0.240	0.624
SOSP 7	0.388	0.533
SOSP 9 *	4.312	0.038 *
SOSP 13	0.166	0.684
SOSP 14	0.000	1.000
Zole B03	0.024	0.876
Zole C02	0.199	0.655

Table 2.2. Standardized heterozygosity (SH) of song sparrows calculated with single-locus dropouts as a predictor of overwinter return, for (a) birds sampled in 2008 and returns assessed in 2009, (b) birds sampled in 2009 and returns assessed in 2010. Removing SOSP 1 or SOSP 7 from the 2008-2009 analysis, and removing Mme 7, SOSP 9 or SOSP 13 from the 2009-2010 analysis, eliminates the observed relationship between SH and return. Removing other loci in some cases causes the relationship to become marginally significant.

a) Locus Removed	**β**	**Wald**	**P**
Mme 1	-2.497	3.125	0.077
Mme 2	-2.560	3.274	0.070
Mme 7	-3.150	4.700	0.030
Mme 12	-3.459	5.228	0.022
Escu 1	-2.266	3.283	0.070
Pdou 5	-2.667	3.501	0.061
SOSP 1	-2.024	2.143	0.143
SOSP 2	-3.317	4.352	0.037
SOSP 3	-2.951	4.095	0.043
SOSP 4	-3.670	6.002	0.014
SOSP 5	-2.647	3.473	0.062
SOSP 7	-2.296	2.687	0.101
SOSP 9	-3.080	4.052	0.044
SOSP 13	-2.792	4.878	0.027
SOSP 14	-2.843	3.919	0.048
Zole B03	-4.877	9.002	0.003
Zole C02	-4.602	7.404	0.007

b) Locus Removed	**β**	**Wald**	**P**
Mme 1	-2.829	3.452	0.063
Mme 2	-3.133	3.655	0.056
Mme 7	-1.780	1.536	0.215
Mme 12	-2.832	2.951	0.086
Escu 1	-3.710	5.015	0.025
Pdou 5	-2.641	2.872	0.090
SOSP 1	-2.936	3.403	0.065
SOSP 2	-3.410	3.698	0.054
SOSP 3	-3.216	4.086	0.043
SOSP 4	-2.712	2.861	0.091
SOSP 5	-3.301	3.938	0.047
SOSP 7	-2.560	2.746	0.097
SOSP 9	-2.251	1.911	0.167
SOSP 13	-2.491	2.630	0.105
SOSP 14	-3.230	3.828	0.050
Zole B03	-2.971	3.360	0.067
Zole C02	-3.055	3.708	0.054

2.4 Discussion

Contrary to my predictions, heterozygosity-fitness correlations were not found at most traits studied. A notable exception was that more genetically diverse males provided more parental care. Unexpectedly, in two of three breeding seasons, genetically diverse males were less likely to return to breed than their less diverse counterparts.

Standardized heterozygosity (SH) did not predict innate immunity as measured by bacterial killing ability of either nestlings or adults (Figures 2.2a, b). This was contrary to my predictions as low genetic diversity has been associated with increased susceptibility to pathogens and infections in many taxa (reviewed above), including my study population (Pfaff et al. 2007, Singh 2007). However, despite a large body of evidence supporting immune-related HFCs in wild populations, other studies have found no relationship (e.g. European eel, *Anguilla anguilla,* Pujolar et al. 2009). Moreover, some studies have concluded that HFCs that were originally thought to be due to global effects (inbreeding depression) are better explained by local effects (Amos and Acevedo-Whitehouse 2009; Banks et al. 2010). Recent criticisms of HFC studies have suggested that heterozygosity may explain less variation in fitness than previously thought and that this field is subject to severe publication bias (Chapman et al. 2009). In my population, bacterial killing ability may be truly unrelated to individual genetic diversity, or there may be local effects (e.g. at immune related loci such as MHC) that were undetectable at the neutral loci I surveyed.

Immune responsiveness has been shown to predict survivorship in Passeriformes (Møller and Saino 2004) and was thus chosen as a proxy for fitness. However, the immune system is complex and it is problematic to use a single assay to study immune function (Adamo 2004; Lee 2006; Liebl and Martin 2009). This bactericidal assay and the strain of *E. coli* were chosen because the assay could be performed using frozen whole blood, and thus was feasible in a field-based study. This laboratory strain of *E. coli* should be novel to this population of song sparrows, and thus should primarily engage the innate immune system (Tieleman et al. 2005), which is particularly relevant to short-lived vertebrates such as song sparrows. Current projects underway in the lab now include multiple immunoassays that should provide a more comprehensive understanding of immune function and how, if at all, it relates to genetic diversity in this population.

The bactericidal assay chosen was appropriate due to its applicability to field studies, its non-invasive approach, and the relatively small blood volume required. It was also attractive because of the biological relevance of the assay: because bacterial infections do occur in the wild, greater bactericidal ability should be associated with increased survivorship or fitness (Leibl and Martin 2009). Conversely, other non-invasive methods of estimating immunocompetence are more difficult to interpret. Early studies assumed that high lymphocyte counts were indicative of robust immune system (Zuk and Johnsen 1998), although this interpretation has been called into question because it could reflect an individual fighting a current infection, having a high immunocompetence, or having an inefficient activation threshold (Kennedy and Nager 2006). Similarly, the magnitude of skin swelling response to the unfamiliar mitogen phytohaemmaglutinin (PHA) has generally been interpreted as indicative of the strength of the innate immune response (Reid et al. 2003), though it has since been shown to induce both innate and acquired immune responses making it difficult to assess results directly (Martin et al. 2006). The bactericidal assay improves on these methods as a more direct and biologically relevant estimate of immune function however; other limitations have recently been discussed. Specifically, freezing plasma severely compromises its killing ability (Liebl and Martin 2009), especially after 20 days in the freezer. All my samples were kept frozen for at least six months, suggesting that their killing ability may have been greatly diminished. However, I found no significant relationship between killing ability and time spent frozen (data not shown) and all samples were frozen for a similar time period. Although this limitation may introduce additional noise into the dataset, it should not introduce substantial bias.

One interesting relationship found using the bactericidal assay was that killing ability differed based on age and sex (Figure 2.3) with females having a significantly higher killing ability than nestlings, and males having an intermediate killing ability. Sex differences in immune function have been studied extensively: testosterone is generally an immunosuppressant, resulting in trade-offs between immunocompetence and many sexual signals in birds (Folstad and Karter 1992; Saino et al. 1995; Duffy et al. 2000; Casto et al. 2001; but see Hasselquist et al. 1998). For this reason, females tend to have higher immunocompetence than males, consistent with my findings. In nestling birds, immunocompetence has been shown to have strong trade-offs with growth rates and survival (Brommer 2004). Thus, immune function during early life may be relatively weak in a species and population with intense nest predation and selective pressure for rapid development and fledging. Within species, nestling immunocompetence varies dramatically with factors including parental feeding rates, parental immunocompetence, age, brood size, temperature, and paternity (Saino et al. 1997; Johnsen et al. 2000; Naguib et al. 2004; Cichon et al. 2006; Garvin et al. 2006; Edler and Friedl 2008; Dunn et al. 2009) as well as with heterozygosity (Fossøy et al. 2008). Across species, nestling immune responses increase with time spent in nest (Tella et al. 2002), and so the low immune response we observed on day 6 after hatch is not surprising. Since the relationship between age and sex and immune response followed our theoretical understanding, it is likely that the results of this bactericidal assay, although compromised by freezing the blood, are still biologically relevant.

Genetically diverse adult birds sampled at the study site in spring 2008 were significantly less likely than their less diverse counterparts to return in 2009, and a similar pattern was found from 2009 to 2010 (Figures 2.4, 2.7). This effect appears to be driven by male return rates (Figures 2.5a, 2.8a). This finding was supported by random resampling from the 2008 dataset demonstrating that these results are very robust. SH of birds that subsequently returned the following spring was significantly lower than SH values generated from a random selection of birds in the 2008, but not 2009 datasets (Figures 2.6, 2.9).

If highly heterozygous males are investing more in current reproduction (as suggested by male visitation rate on day 6, Figure 2.17b), they may be exhausting their energetic resources and failing to return to breed the following season as a result. Since breeding philopatry is very high in this species (Nice 1941; Greenwood 1982; Clarke et al. 1997) and population, we routinely assume that most individuals that do not return to the site have died overwinter (e.g. MacDougall-Shackleton et al. 2009). Together, these findings suggest there may be a trade-off between investment in current and future reproduction, where more heterozygous males invest heavily in current reproductive success at a cost to future reproductive success. Trade-offs between different components of fitness or between current versus future reproduction are well documented in the literature (Coleman et al. 1985; Møller 1994, 1997; Norris et al. 1994; Brommer 2004). For example, intense reproductive investment in great tits (*Parus major*) has been correlated with poor health status (Ots and Horak 1996). The authors propose that this is due to a low future reproductive potential (less than 50% of adults return to breed in subsequent years, a figure similar to that observed in my study population), making a terminal investment strategy optimal for many individuals. Similarly, if highly heterozygous male song sparrows invest more in current offspring, they may be less able to invest in migration and maintenance.

Interestingly, the negative relationship between SH and overwinter return rates was not observed in birds breeding in 2007 and returning in 2008, for either males or females (Figures 2.10, 2.11). Variation across years in selective pressures could cause this difference, for example if weather, resource availability, and risk of disease or predation fluctuate substantially between years. Since return rates vary yearly, this is a strong possibility (38% - 47% return observed from 2007-2010). The survival consequences of high levels of parental investment may thus vary from year to year. This possibility can be viewed in light of game theory, which predicts that multiple strategies may exist stably within a single population (Maynard Smith 1982). For example, aggressive risk-takers with high energy consumption and cautious risk-avoiders that conserve energy may encounter different costs and benefits depending on environmental, seasonal, and population contexts (Korté et al. 2005). Thus, genetically diverse males who invest highly in the current reproductive attempt may incur survival costs in some years, but not in other years. Future longterm studies, investigating return rates over many years would help to answer the questions posed by this study. More rigorous studies using radio telemetry to track adults during migration would improve our understanding of breeding philopatry, and test the assumption that individuals fail to return have died, would also strengthen interpretations of these results.

SH did not predict nestling growth rates during the time period examined (Figure 2.13). This was surprising because previous work in this population demonstrated that nestlings of genetically dissimilar parents (thus, presumably genetically diverse themselves) grew faster (Potvin and MacDougall-Shackleton 2009). That previous work also found that females invested more in offspring when paired with genetically dissimilar males, which could amplify apparent HFCs in nestling growth rates (Potvin and MacDougall-Shackleton 2009). As I found no relationship between nestling genetic diversity and growth, parental investment may be the main factor responsible for the pattern observed by Potvin and MacDougall-Shackleton (2009). My sample size was substantially smaller than that of the previous study and would be insufficient to detect very weak effects (as considered most common by Chapman et al. 2009), but I had very strong power to detect effects of the magnitude previously reported (Potvin and MacDougall-Shackleton 2009). Thus, it seems ecological differences should account for these results. Theory predicts that when genetic diversity is positively related to survivorship, adults should be more genetically diverse than juveniles, due to early-life selection against the least fit (homozygous) genotypes (Cohas et al. 2009). In this scenario, heterozygosity should be most strongly related to fitness in juveniles. In contrast, I found greater genetic diversity among nestlings than among adults (Figure 2.13), possibly due to highly heterozygous adults being less likely to return in 2009 (Figure 2.4). These findings, combined with the finding that SH did not predict bacterial killing in adults or nestlings suggest that genetic diversity is not positively related to nestling survival in this population.

SH did not predict any of the measured indices of reproductive success (Figures 2.14-2.16). Males that were more genetically diverse were somewhat less likely to lose paternity or to have their nests fail, and females that were more genetically diverse appeared to have larger clutches: however, these relationships were not statistically significant. In our large and non isolated study population, the risk of inbreeding is likely to be low. Thus, even the least genetically diverse individuals may still not suffer any costs in terms of reproductive success.

Moreover, in a population where fledging success depends almost entirely on avoiding predation (as it does in my study birds), territory quality and nest defense probably explain much more variation in reproductive success than does genetic quality (including the nonadditive effects investigated here). In many animals, heterozygosity is associated with enhanced disease resistance, developmental stability, condition and attractiveness (Brown 1997), all traits which might be expected to provide heterozygous males with a mating advantage. However, because heterozygosity is not itself heritable (except in specific conditions, see Reid et al. 2006; Neff and Pitcher 2008), these advantages are generally not transmitted to offspring. Thus, if there are any positive relationships (which have not been uncovered thus far), females may be better off choosing genetically dissimilar, rather than genetically diverse mates; this possibility is explored in Chapter 3. The lack of relationships between female genetic diversity and reproductive success is expected, given that most HFCs are more prevalent in the competitive sex (Merilä et al. 2003).

SH was positively associated with male parental care on day 6 after hatching (Figure 2.17b) although not on day 2 after hatching (Figure 2.17a). Song sparrows require a high level of biparental care (Smith et al. 1982) and both sexes provide essential care for the offspring until they reach independence (Hochachka and Smith 1991). This investment is the final determinant of a parent's reproductive success (Neff and Pitcher 2005), as investment in offspring can have important fitness consequences (Nowicki et al. 1998). The lack of relationship early in the nestling period is perhaps not surprising, because males only rarely visit the nest at this stage. On day 2, most feeding and all thermoregulating is done by females. In contrast, paternal care is more important later in the nesting cycle, with visits by females decreasing and by males increasing throughout the nestling period (data not shown). Thus, when males are contributing more to parental care, heterozygous males are contributing the most. This finding lends support to the theory of mate choice for heterozygosity (Brown 1997) because females could gain substantial benefits from choosing heterozygous males. Nestling condition and nutrition has carry-over effects later in life (Searcy et al. 2004), such that better parents may have enhanced fitness through more successful offspring.

Female heterozygosity was not associated with either the number of nest visits or the proportion of time spent incubating (Figures 2.18, 2.19), suggesting that maternal investment may be independent of female genetic diversity. In fact, most studies of maternal investment have focused on variation in motivation (e.g. increased investment in young when paired with highly ornamented (Burley 1986, 1988) or genetically dissimilar males (Potvin and MacDougall-Shackleton 2009) rather than variation in ability to provide care. These ideas may be more relevant to female investment in offspring and will be discussed in the following chapter.

The heterozygosity-heterozygosity test (Balloux et al. 2004, Lieutenant-Gosselin and Bernatchez 2006) demonstrated that heterozygosity at microsatellite loci was not correlated across the genome. This suggests that despite the large number of loci used in this study, standardized heterozygosity in this population, like many others, may not reliably estimate the inbreeding coefficient. A test for local effects, which therefore may be more appropriate, revealed that the relationship observed between male genetic diversity and the likelihood of returning was strongly driven by a single microsatellite locus, SOSP 1 in 2008 (Table 2.1a) and SOSP 9 in 2009 (Table 2.1b). Another test, which evaluates local effects by omitting each locus one at a time from SH calculations demonstrated that other loci (SOSP 7 in 2008, and Mme 7 and SOSP 13 in 2009) were also contributing to the negative relationship (Table 2.2a, b). Several other studies have recently found support for local effects (across five fitness components in threespine stickleback, *Gasterosteus aculeatus,* Lieutenant-Gosselin and Bernatchez 2005; cell-mediated immunity in bluethroat nestlings, *Luscinia s. svecica,* Fossøy et al. 2009; adult survival in possums, *Trichosurus cunninghami,* Banks et al. 2009; recruitment and survival in great-reed warblers, *Acrocephalus arundinaceus,* Hansson et al. 2001 and 2004) driving HFCs.

There are limitations to this method of studying parental care and investment. PIT readers record visits by each tagged adult, but not the nature of their visit. We assume that most visits at this stage involve delivering food, but they could also be to check on nestlings, thermoregulate, defend, or repair nests. However, as all of these types of visits are forms of care, this should not bias our interpretation substantially. A previous study of this population found that the most visited nestlings grew almost 15% faster than the least visited nestlings (Potvin and MacDougall-Shackleton 2009), suggesting that regardless of the exact proportion of visits that are associated with feeding, more parental visits are associated with enhanced nestling performance. Another and more serious limitation was the small sample size for which visitation rates were available.

The relationship between heterozygosity and fitness in the population appears to be more complex than was originally hypothesized. Contrary to my original prediction, individual genetic diversity did not predict bacterial killing ability (an index of innate immune function), nor was it related to nestling growth rates or to most of the measures reproductive success that I examined (with the exception of paternal care). In two years, there was an unexpected negative relationship between heterozygosity and overwinter return rates of breeding males. This relationship was primarily explained each year by a few microsatellite loci, potentially implicating local rather than genome-wide effects. However, the specific loci implicated differed between years. Sequencing the flanking regions of the loci involved in overwinter return might help to identify nearby coding regions that contribute to the strikingly lower apparent survivorship of heterozygotes at these loci.

The increased parental investment by heterozygous males suggests that genetically diverse individuals may focus their energy on the current breeding season, at the expense of future survival. This trend may be dependent on a variety of environmental factors and, like many other evolutionarily stable strategies, is likely to fluctuate. Following this population for several years will provide further insights into the nature of these relationships.

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Chapter 3: Genetic compatibility and mating strategies in song sparrows (*Melospiza melodia*)

3.1 Introduction

Early models of mate choice (e.g. Zahavi 1975; Andersson 1994) focused on elaborate ornaments and courtship displays and proposed that females prefer males with elaborate traits because they signal good genes that can be passed to offspring. In this scenario, female preferences should be universal, and the most highly ornamented males should have the highest reproductive success. However, Trivers (1972) first proposed that females may choose males that best complement their genetic makeup. Recent theoretical and empirical work suggests that female mating preferences may sometimes be idiosyncratic rather than congruent. In light of the costs of inbreeding and conversely, the advantages of genetic diversity apparent in many species (reviewed in Chapter 2), models of sexual selection have expanded to consider the possibility that individuals may select mates based in part on genetic dissimilarity (Trivers 1972; Pusey and Wolf 1996; Tregenza and Wedell 2000; Mays and Hill 2004; Neff and Pitcher 2005).

Widespread testing of these "genetic compatibility" models has recently become possible due to advances in molecular techniques (Zeh and Zeh 2003). In bluegill sunfish (*Lepomis macrochirus*), for example, mating interactions involving female choice approached an optimal level of parental genetic divergence, while opportunistic matings were further from the optimal range (Neff 2004). More extreme examples of mating for genetic compatibility come from plants; in angiosperms, self-incompatibility obliges some species to avoid self-fertilization (e.g. *Arabidosis lyrata,* Charlesworth et al. 2003). The importance of genetic compatibility is likely to vary substantially between taxa and populations and to depend on a variety of factors. In highly structured kin populations that experience severe inbreeding depression, the benefits of mating disassortatively are more likely to outweigh the assessment costs than in populations where genetic diversity is less closely tied to individual fitness, as females may have little to gain from choosing dissimilar mates (Mays and Hill 2004). Finally, in populations subject to pathogen pressure, females may benefit through disassortative mating if heterozygote advantage confers disease resistance, but not if disease resistance is associated primarily with 'good genes' and predicted by ornamentation (Mays and Hill 2004). These examples illustrate how the benefits of disassortative mating are unlikely to be uniform among, or even within, species.

Population genetic structure and history, and thus the relative costs of inbreeding, are likely to influence whether or not inbreeding avoidance is a key mating tactic for any given population (Mays et al. 2008). Moreover, even if inbreeding avoidance or mate choice based on complementary genes would be adaptive, taxa may differ in their ability to assess the genetic similarity of a potential mate. In order to mate disassortatively, females must be able to compare their own genetic makeup to that of potential mates, or use a proxy method to assess genetic similarity (Mays et al. 2008, but see Pusey and Wolf 1996 for other inbreeding avoidance mechanisms such as delayed maturation and sex-biased dispersal). In a review of the evidence for heterozygote advantage and mate choice for genetic compatibility in birds, Mays et al. (2008) and reported widespread heterozygote advantage in sexually selected traits such as song complexity and territory size, as well as increased pathogen resistance and immunocompetence (discussed in Chapter 2). However, despite the apparent importance of producing genetically diverse offspring, the authors found little evidence that most birds select mates based in part on genetic complementarity.

Mays et al. (2008) suggest that mate choice in birds is a behaviourally plastic process that can change throughout the breeding season, between populations, and across species. Females may choose to pair socially with males that can secure the best territory and provide direct benefits, and then produce extra-pair young with a genetically dissimilar mate. In this way, females may 'trade up' or select extra-pair mating partners based on their genetic compatibility (Jennions and Petrie 1999; Johnsen et al. 2000). Depending on female needs and male availability, mate choice may be plastic and vary between choice for good genes and choice for compatible genes (Mays et al. 2008). In a taxonomically broader review, however, Neff and Pitcher (2005) argued that while conclusive evidence for genetic compatibility affecting mate choice is rare, the few studies that demonstrate a compatibility based mate choice strategy suggest that genetic compatibility may be more important to mate choice and variance in fitness than good genes. Both reviews emphasize the necessity for further studies of genetic compatibility and its effects on fitness as well as on mate selection.

In birds, the most consistent evidence for mate choice for compatibility involves choice of extra-pair mates (Mays et al. 2008). Females in many species appear to choose their social mates for direct benefits such as better territories, parental care, or nest defence, and 'trade up' for genetic compatibility when selecting extra-pair mates. Indirect support for this idea comes from the splendid fairy wren (*Malurus splendens*), where frequent social pairing between kin is observed but most offspring are the result of extra-pair matings and sired by unrelated mates (Brooker et al. 1990). In three species of shorebirds (Blomqvist et al. 2000) as well as Mexican Jays (*Aphelocoma ultramarine,* Eimes et al. 2005), and Savannah sparrows (*Passerculus sandwichensis,* Freeman-Gallant et al. 2003), band sharing or relatedness between social parents predicted incidence of extra-pair offspring. These divergent preferences for social versus extra-pair males support hierarchical (Neff and Pitcher 2005) or individual optimization models (Freeman-Gallant et al. 2003) where females may choose the most dissimilar mate among those with good genes, or the best male among those that are genetically compatible, depending on the selective pressures to which they are subject.

A recent study of song sparrows (*Melospiza melodia melodia*) demonstrated that females mated to genetically dissimilar males visit nests significantly more often than those mated to genetically similar males (Potvin and MacDougall-Shackleton 2009). A similar trend was found for male investment though it was not statistically significant, and offspring of genetically dissimilar parents grew faster than those of similar parents. These findings suggest that an advantage which at first appears to be caused entirely by nonadditive genetic effects may be amplified by preferential investment in outbred offspring (Potvin and MacDougall-Shackleton 2009). Certainly, this study provides support for behavioural consequences of parental genetic dissimilarity in this population, but leaves room for more work, as only 7 microsatellite loci were used.

My objective in this chapter is to determine whether genetic compatibility affects social and/or extra-pair mate choice and parental investment in song sparrows. Heterozygote advantage in this population is mixed (see Chapter 2) and appeared to be limited to parental ability in the 2009 breeding season. In other years however, heterosis has been found in nestling growth and parasite load, such that if these benefits confer a substantial fitness advantages, song sparrows should mate disassortatively. To test the hypothesis that social mate selection is affected by genetic similarity, I investigated whether socially mated pairs are less genetically similar to their mate (as assessed by neutral-locus similarity at 17 microsatellites) compared to all other opposite sex adults in the population. Based on the empirical finding that mate choice for genetic compatibility is often observed in extra-pair mating systems (Mays et al. 2008) and the fact that high rates of nest predation likely makes direct benefits such as territory quality very important, mate choice for compatibility may be primarily expressed through extra-pair mating tactics. Thus, I also investigated whether females paired with genetically similar social mates were more likely to produce extra-pair young. Finally, I investigated whether or not parental investment (clutch size and nestling provisioning) varies with genetic similarity between social mates. Parental relatedness may also affect fledging success, but because most nests that fail are caused by snake predation (which song sparrows are largely unable to prevent), measures such as clutch size and feeding rates may prove more informative about parental investment.

3.2 Methods

Field methods

All field work was conducted on land owned by the Queen's University Biological Station, near Newboro, ON (44° 38' 60 N / 76° 19' 0 W) between April 11th and June 25th, 2009. Adult song sparrows were captured as described in Chapter 2, and fitted with a unique combination of colour bands, a Passive Integrated Transponder (PIT) tag, and a Canadian Wildlife Service (CWS) band for identification and nest monitoring. A small blood sample was taken for future genetic analyses. I identified social pairings, found nests through behavioural observations, and monitored them until nestlings hatched. On days 2 and 6 after hatch, I placed PIT detectors at the nest as described in Chapter 2, to record parental nest visits. I collected small blood samples from each nestling on day 2 after hatch for genetic (parentage) analysis, as described in Chapter 2. Each nest was then monitored as described in Chapter 2 until it either produced fledglings or failed, usually due to predation.

Genetic methods

Genomic DNA was extracted following Laitinen et al. (1994) and was amplified at 18 microsatellite loci, 17 of which were variable in the study population (see Chapter 2 Methods) using the polymerase chain reaction (PCR). PCR products were sized using an Applied Biosystems 3130 Genetic Analyzer and scored manually using the software Gene Mapper. Detailed genetic methods can be found in Chapter 2.

All loci were checked for null alleles as described in Chapter 2. Loci harbouring null alleles were excluded from parentage analysis but all 17 loci were included in generating pairwise relatedness coefficients. A review of how null alleles are handled in the scientific literature (233 studies) concludes that most instances of null alleles are unlikely to introduce serious bias (especially when frequencies of null alleles are <0.2, and multiple microsatellite loci are used) and the vast majority of studies state putative frequencies rather than correct for this problem (Dakin and Avise 2004). Similarly, Blouin concludes that null alleles rarely introduce substantial bias to relatedness analyses (2003).

Parentage analysis

To assess paternity, I compared the microsatellite profiles of nestlings to those of their social parents at six microsatellite loci (SOSP 13, SOSP 3, SOSP 14, Mme 2, Escμ1, Pdoμ5). All nestlings had genotypes consistent with those of their social mothers. With known maternity, Cervus 3.0 predicted a >99.99% exclusion probability of paternity for this dataset (Kalinowski et al. 2007). In cases of at least one mismatch between offspring and social father, an additional panel of microsatellite profiles was used (SOSP 9, SOSP 2, SOSP 4, Mme 1) to confirm exclusion. In all cases, nestlings identified as extra-pair offspring mismatched their social father at two or more of the ten loci screened. I attempted to identify the genetic fathers of any extra-pair offspring based on the microsatellite profiles of the other males in the population, but in most cases none of the males genotyped had microsatellite profiles consistent with those of extra-pair offspring, suggesting that the genetic sires of such offspring were either transient or held territories outside of the study area.

Similarity analysis

I calculated the genetic similarity of every adult sampled in the population to every other adult sampled based on their microsatellite profiles using MARK relatedness software (Ritland 1996). This program generates Wang's (2002) pairwise coefficient of relatedness. In theory, Wang's coefficient of relatedness ranges from -1 (very dissimilar individuals) to +1 (identical individuals). In the study population, values of Wang's r ranged from -0.343 to 0.418 among breeding adults. To correct for an individual's similarity to the population as a whole, I subtracted its average relatedness to all potential mates (opposite sex adults) in the population from the relatedness to its social mate. I then compared this "corrected relatedness" to several indices of mating and parental care behaviour.

Statistical analyses

To investigate social mating strategies, I conducted a one-sample t-test to evaluate the hypothesis that corrected relatedness between individuals and their social mates was significantly different from 0. To investigate whether similarity of social mates predicts the likelihood of a male retaining paternity, I used a logistic regression. Clutch sizes ranged from 4-6 in this study, but because only one individual laid 6 eggs and a linear regression was inappropriate, I characterized clutch sizes as 4 or 5+ and performed a logistic regression.

I measured parental care separately for males and females, on days 2 and 6 after hatching. For males, I calculated the rate of nest visitation (visits per hour per nestling). I did the same for females but also calculated time spent at the nest per hour as females also brood nestlings before they can thermoregulate on their own. I used linear regressions to analyze the relationships with corrected relatedness as the independent variable and visits/nestling/hour on each day and for each sex as the dependent variables. Preliminary models included date and weather conditions (characterized as "fair" or "rainy") as independent variables, but neither of these explained a significant proportion of variation in the dependent variables examined (data not shown) so the results presented below do not include date and weather in the models. All variables were tested for normality using the Kolmogorov-Smirnov test and visual inspection, and were found to be normally distributed. All analyses were done on SPSS Statistics Student Version 17.0.1 (2008) or PASW Statistics Version 18 (2009). All statistical tests were two-tailed.

3.3 Results

Genetic similarity and social mate choice

The average pairwise relatedness of all adults in the study population was -0.026, with a normal distribution ranging from -0.343 to 0.418 (Figure 3.1a). Coefficients of relatedness between pairs of social mates had a mean of -0.021 and ranged from -0.168 to +0.177 (Figure 3.1b). For some perspective, a subset of nestlings (34 nestlings from 10 families) known to be full siblings were analyzed and the average Wang's coefficient of relatedness within a nest group was 0.246

(sibling relatedness ranged from 0.163 to 0.361). A similar analysis using extra-pair offspring and their within-pair maternal half-siblings (15 individuals from 5 families) showed that average half-sibling relatedness was 0.158 (half-sibling relatedness ranged from 0.063 to 0.250).

Corrected relatedness (defined as relatedness to mate – relatedness to all opposite sexed individuals in the population) of all socially paired individuals was not significantly different from 0 (Figure 3.2. one sample t-test, $t_{41} = 6.34$, $p = 0.529$). This lack of relationship was consistent whether a female choice model, using female's corrected relatedness to her social mate ($t_{20} = 0.286$, $p = 0.778$), a male choice model, using male's corrected relatedness to his social mate ($t_{20} = 0.617$, $p = 0.544$), or an uncorrected model that used Wang's pair relatedness without accounting for population relatedness ($t_{20} = -1.277$, $p = 0.216$) was used.

Genetic similarity and extra-pair mating tactics

Females that were more similar to their social partners were more likely to produce extra pair offspring (Figure 3.3, $\beta_{20} = 67.410 \pm 32.629$, Wald = 4.268, $p = 0.039$).

Genetic similarity and parental investment

Corrected relatedness to social mates did not predict likelihood of fledging offspring for either males (Figure 3.4a., $\beta_{20} = -2.435 \pm 6.078$, Wald = 0.187, $p = 0.727$) or females (Figure 3.4b., $\beta_{20} = -4.830 \pm 6.515$, Wald = 0.550, $p = 0.458$). Similarly, corrected relatedness of females to their social mate did not predict the number of eggs laid (Figure 3.5. $\beta_{20} = 1.677 \pm 6.390$, Wald <0.069, $p = 0.793$).

Corrected relatedness between females and their social mates did not predict maternal visits (Figure 3.6a., $\beta_{14} = 37.611 \pm 23.403$, $r^2 = 0.166$, $p = 0.132$), or visits per nestling (Figure 3.6b., $\beta_{14} = 10.944 \pm 10.301$, $r^2 = 0.08$, $p = 0.307$), or time spent at the nest (Figure 3.6c., $\beta_{14} = 3535.848 \pm 2730.939$, $r^2 = 0.195$, $p = 0.220$) on day 2.

On day 6, I observed a negative trend between female corrected relatedness and nest visits, although this only approached statistical significance (Figure 3.7a., $\beta_8 = -58.157 \pm 26.536$, $r^2 = 0.407$, $p = 0.065$) and was not observed for visits per nestling (Figure 3.7b., $\beta_8 = 3.294 \pm 7.825$, $r^2 = 0.025$, $p = 0.686$). Also on day 6, I observed a positive trend between female corrected relatedness and time spent at nest, but this was not statistically significant (Figure 3.7c., $\beta_8 = 11428.11 \pm 5896.771$, $r^2 = 0.349$, $p = 0.094$).

In males, corrected relatedness to social mates was not related to visitation rates (Figure 3.8a., $\beta_8 = -10.249 \pm 17.466$, $r^2 = 0.047$, $p = 0.576$) or visitation rates per nestling (Figure 3.8b., $\beta_8 = 3.823 \pm 3.326$, $r^2 = 0.159$, $p = 0.288$) on day 2. Similarly on day 6, I observed no relationship between male corrected relatedness and either visitation rate (Figure 3.9a., $\beta_6 = -33.935 \pm 23.810$, $r^2 = 0.289$, $p = 0.213$) or visitation rate per nestling (Figure 3.9b., $\beta_6 = 1.997 \pm 6.191$, $r^2 = 0.020$, $p = 0.760$).

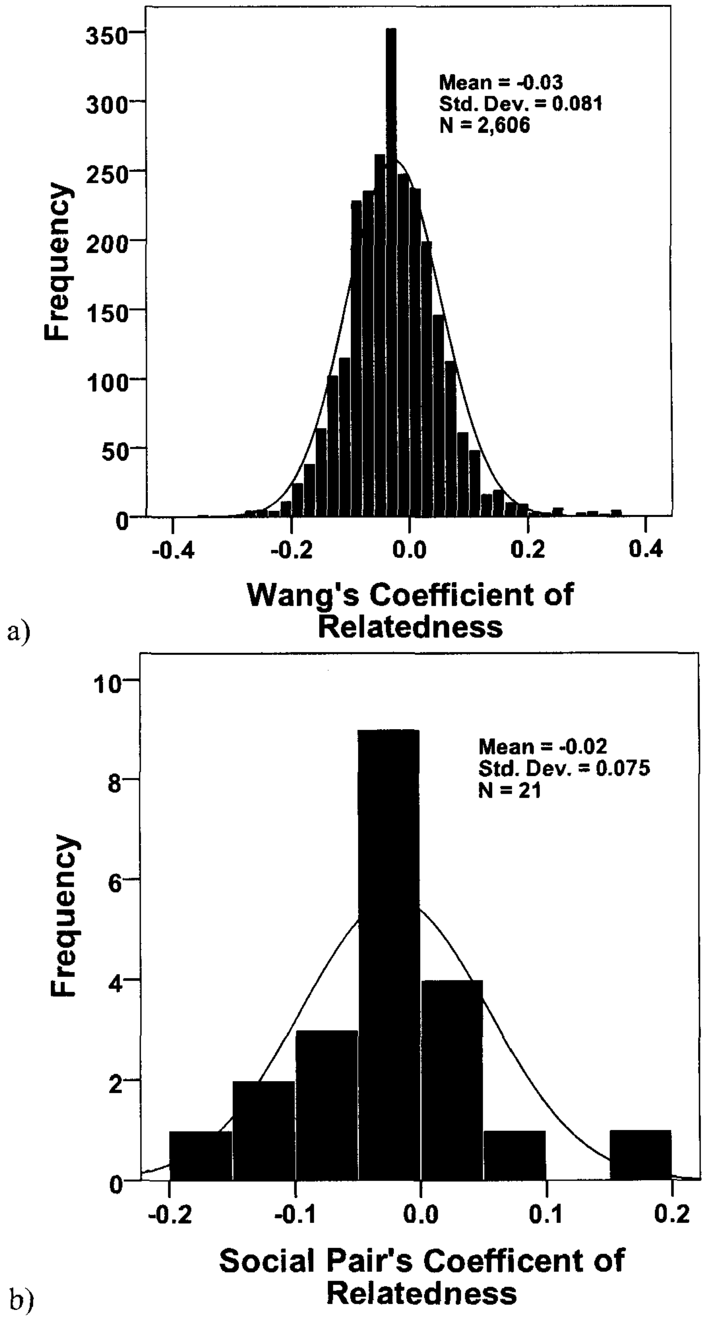


Figure 3.1. Frequency distributions of Wang's (2002) coefficient of relatedness of song sparrows sampled in 2009 for a) all pairwise combinations of breeding adults sampled in 2009 (N = 72 adults, 2606 pairwise comparisons) b) all socially mated pairs sampled in 2009 (N = 21 pairs).

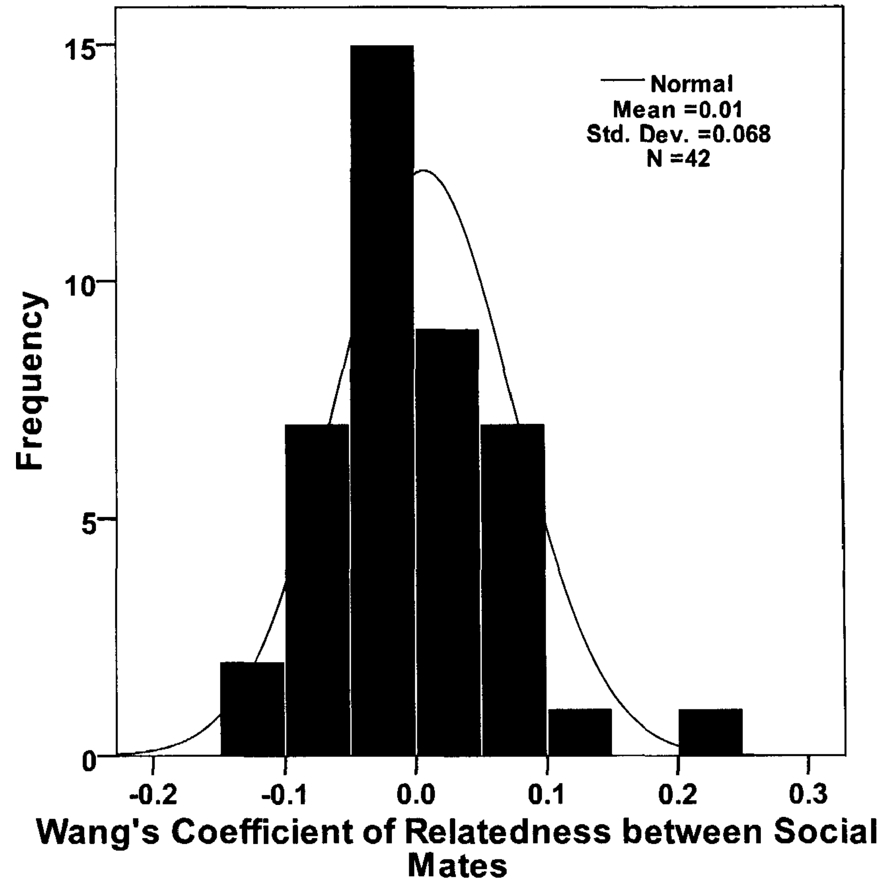


Figure 3.2. Frequency distribution of Wang's coefficient of relatedness of socially paired song sparrows sampled in 2009. The corrected relatedness of social mates did not differ significantly from their relatedness to the rest of the population.

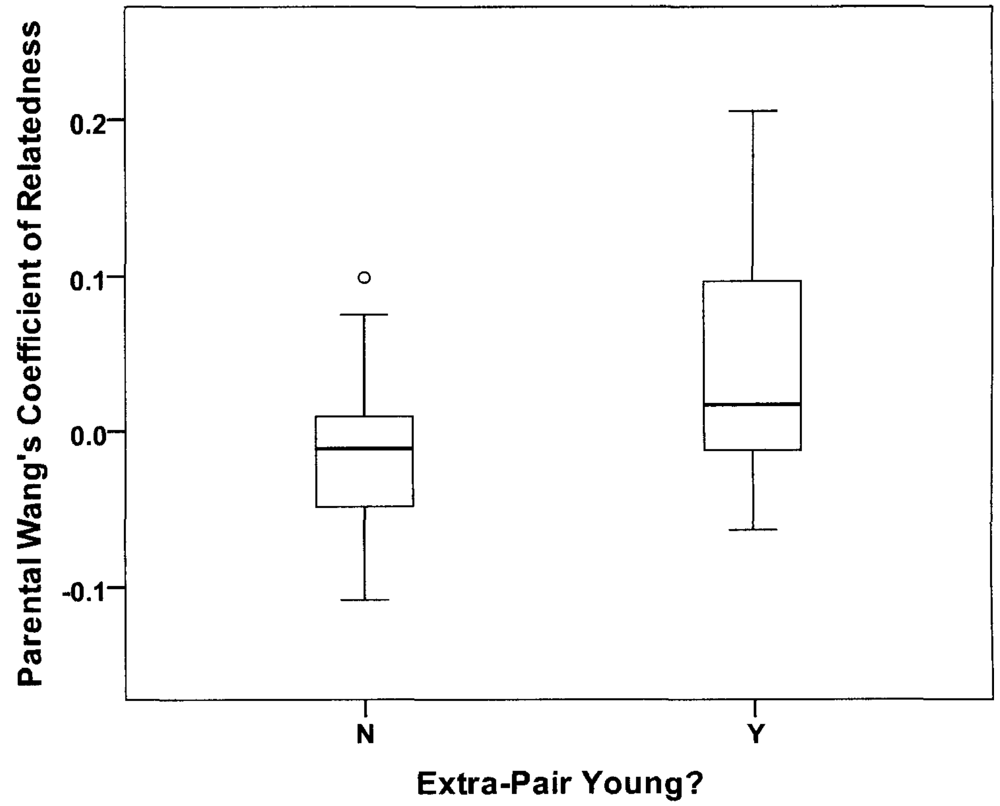


Figure 3.3. Presence of extra-pair young in song sparrow nests sampled in 2009 as a function of social pair relatedness. Females mated to genetically similar males were more likely to have extra-pair young (N = 21). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

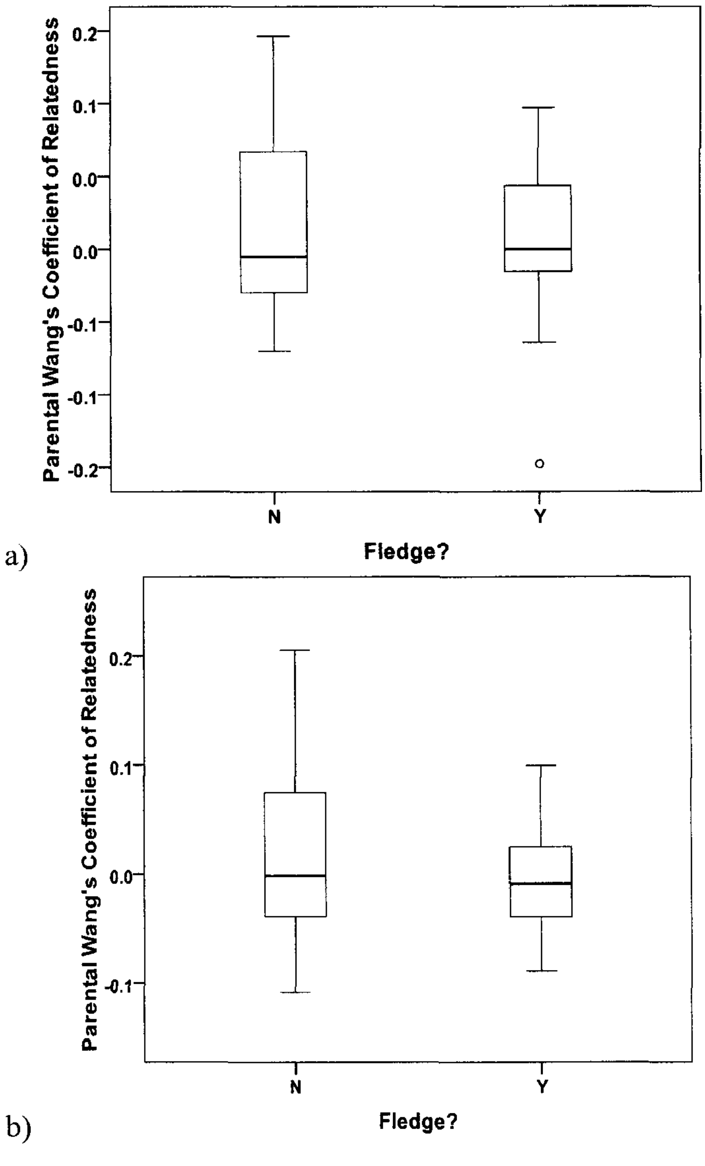


Figure 3.4. Fledging success as a function of parental Wang's coefficient of relatedness in song sparrows sampled in 2009. The corrected relatedness of social pairs does not predict likelihood of fledging for a) males (N = 21) or b) females (N = 21). Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Open circles represent points outside 1.5 x IQR.

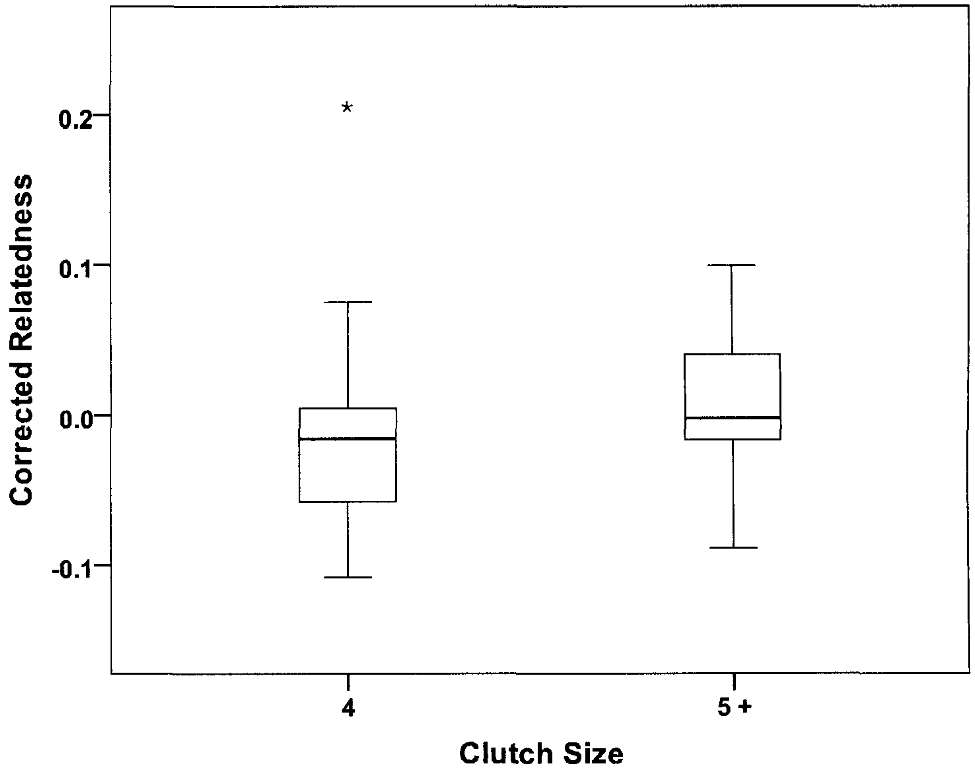


Figure 3.5. Clutch size as a function of parental corrected relatedness in song sparrows sampled in 2009. Female relatedness to her social mate does not predict clutch size. Boxes represent the 75th, 50th, and 25th percentiles. Whiskers represent 1.5 x the interquartile range (IQR), or the maximum and minimum values when these fall within 1.5 x IQR. Asterisks represent points outside 3 x IQR.

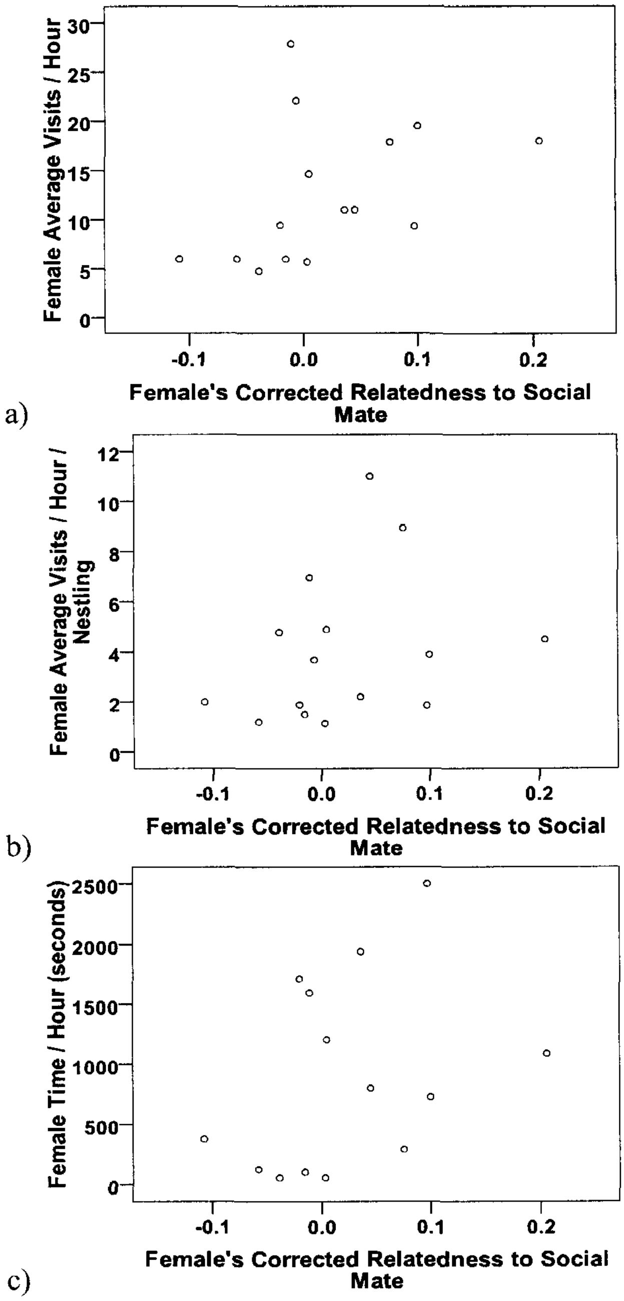


Figure 3.6. Nest visitation times and rates of female song sparrows sampled in 2009 as a function of Wang's corrected relatedness to social mate. The corrected relatedness of females to their social mate did not predict a) nest visits/hour, b) nest visits/hour/nestling, c) time spent at nest/hour (seconds) on day 2 (N = 15).

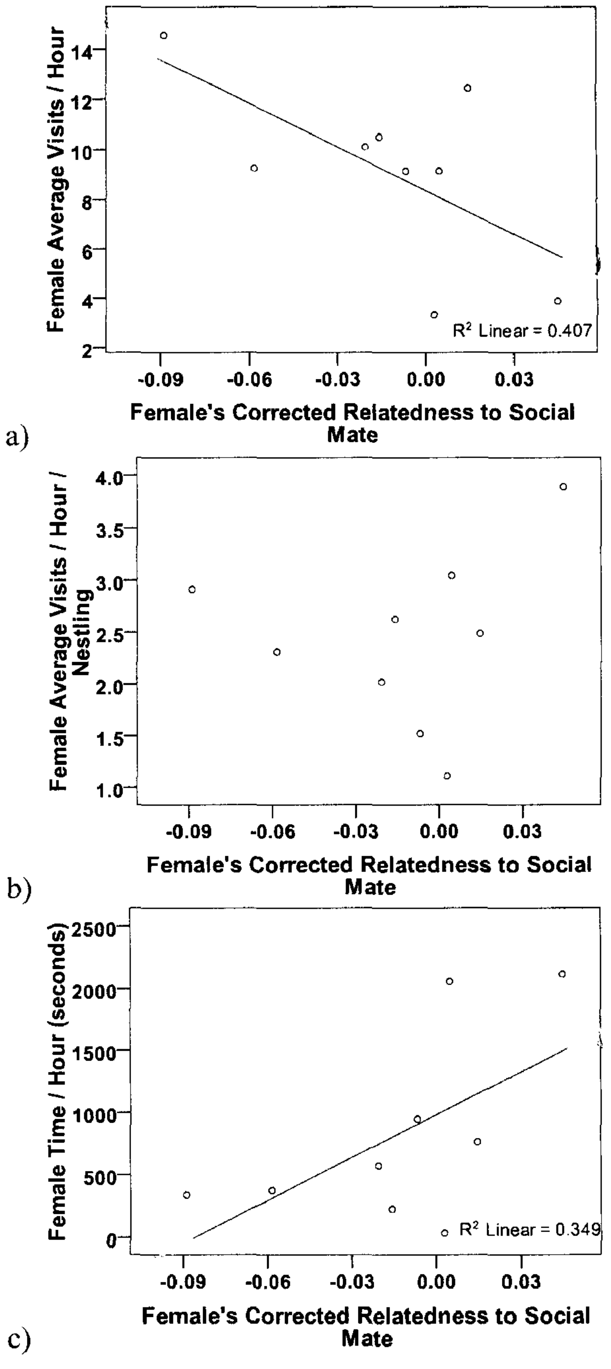


Figure 3.7. Nest visitation times and rates as a function of corrected relatedness in female song sparrows sampled in 2009. Genetic similarity between social mates did not predict female a) nest visits per hour, b) nest visits/hour/nestling, or c) time at nest/hour on day 6 (N = 9).

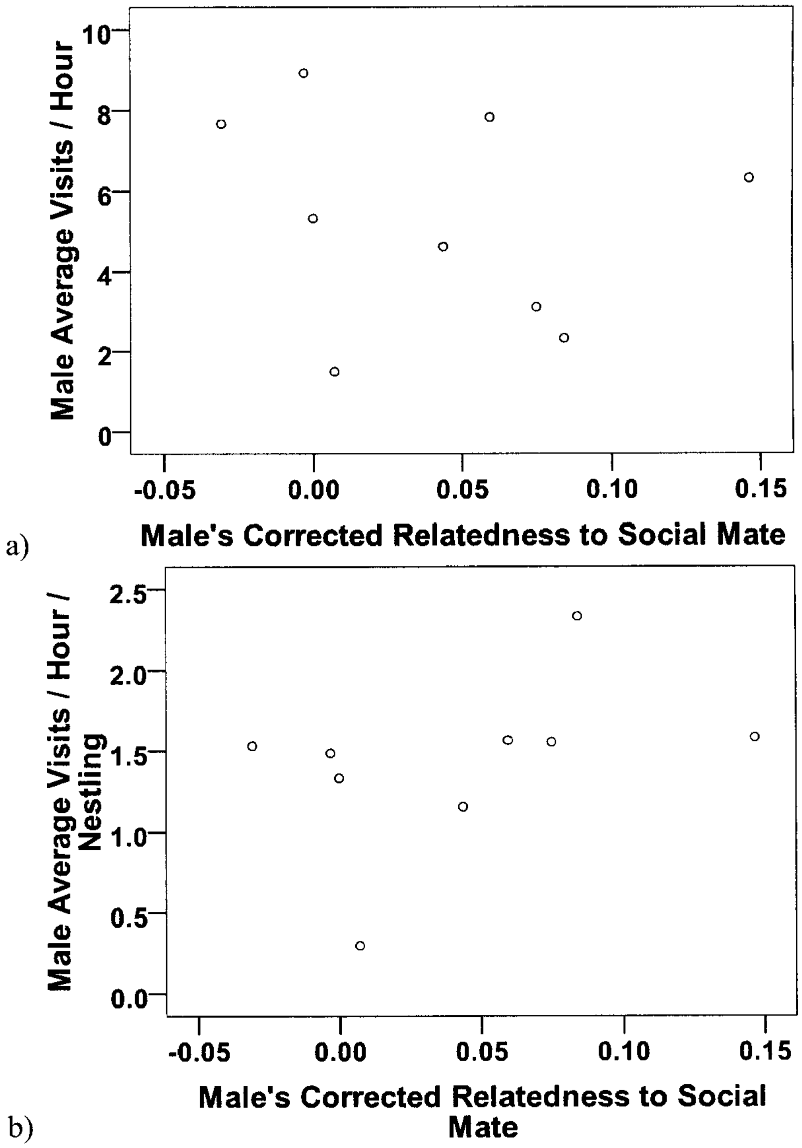
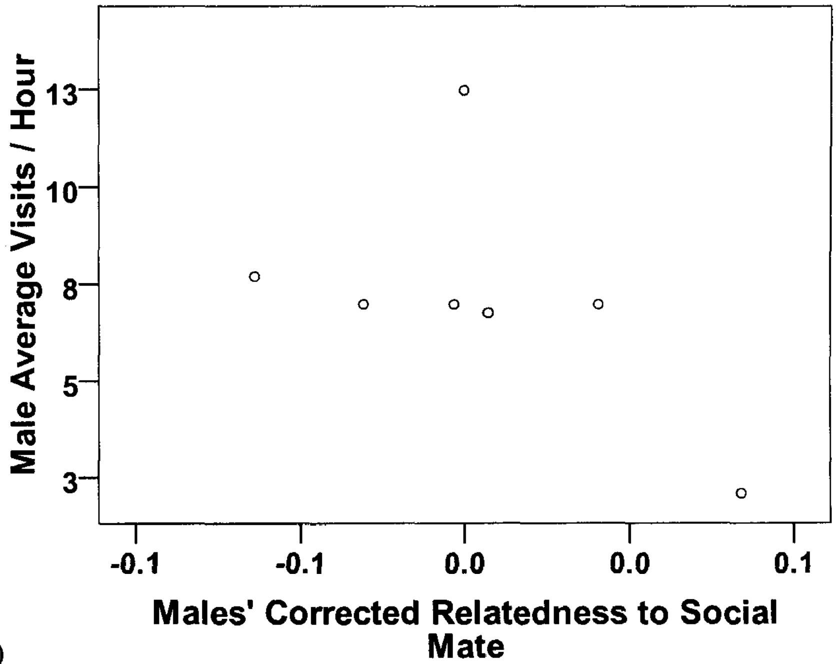
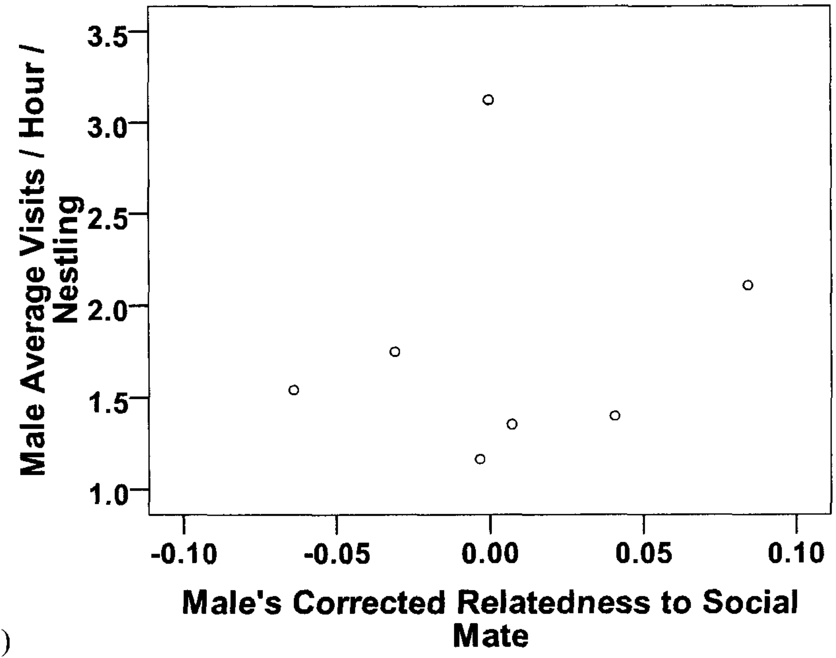


Figure 3.8. Nest visitation rates as a function of relatedness to social mate in male song sparrows sampled in 2009. Corrected relatedness of males to their social mates does not predict a) paternal visits per hour or b) paternal visits per hour per nestling, on day 2 (N= 8).



a)



b)

Figure 3.9. Male visitation rates as a function of corrected relatedness to social mate in song sparrows sampled in 2009. Corrected relatedness of males to their social mates does not predict a) paternal visits per hour or b) paternal visits per hour per nestling, on day 6 (N = 8).

3.4 Discussion

The genetic similarity of adult song sparrows on our Bracken field site has a larger range (Figure 3.1a) than the genetic similarity of social pairs (Figure 3.1b) suggesting that both extremely dissimilar and extremely similar individuals are unlikely to pair as social mates. Comparing the average full sibling relatedness (0.238) to the highest observed relatedness of social mates (0.177), it seems that song sparrows in this population may perhaps avoid mating with very close kin at most (siblings, parents, offspring) but not with slightly more distant relatives. A subset of social pairs fall within the relatedness range of half-siblings, suggesting that kin discrimination is crude at best. Similarly, Keller and Arcese (1998) found no evidence for inbreeding in a population of song sparrows that is subject to high inbreeding depression. They proposed that the costs of forgoing a mating opportunity are higher than the costs of inbreeding.

Song sparrows in this population appear to select social mates at random with respect to genetic similarity (Figure 3.2). Given that most nest attempts in 2009 failed due to nest predation (detailed below), consistent with a minimum of 50% nest failure observed in this population from 2007-2010, females are likely to be selecting social mates based on direct, rather than indirect benefits. Securing a social mate with a high quality territory (for example, one with good ground cover or relatively few predators) may be more important than securing a social mate with compatible genes. In an extensive review of avian mate choice for genetic compatibility, Mays et al. (2008) agree that the potential benefits of mating disassortatively may be outweighed by the benefits of choosing social mates based on direct benefits.

Interestingly, although I found no evidence that females avoid pairing socially with genetically similar males, such females were significantly more likely produce extra-pair young (Figure 3.3). This finding suggests that females have different selection criteria for social versus extra-pair mates. 'Trading up' through extra-pair copulations has been proposed as a solution to why females may mate with extra-pair males in addition to their social mate (Jennions and Petrie 1999). If access to direct benefits is limiting, as it often is in species like song sparrows that require bi-parental care (Møller 1992), then extra-pair copulations provide access to preferred males' genes while maintaining access to their social mate's direct benefits. Early studies assumed that such 'trading up' is done primarily to obtain additive good genes for offspring, but my findings add to a growing body of evidence suggesting that females may also 'trade out' for genetic compatibility or to avoid the costs of inbreeding. Mays et al. (2008) reviewed evidence for mate choice based on genetic compatibility and found that although heterozygote advantage is widespread in traits such as song, plumage coloration, territory size, and immunocompetence, evidence that female birds actually select mates based on genetic complementarity is weak. The authors suggest that mate choice is behaviourally plastic and can change throughout the breeding season, between populations, and across species. Females may choose to pair socially with a male that can secure the best territory and provide direct benefits, but produce extra-pair young with a genetically dissimilar mate. Thus, the relative importance of direct benefits, good genes and compatible genes may differ between populations, between species and depending upon the breeding context (Mays et al. 2008).

The importance of genetic compatibility may be underestimated in this and other populations if incompatible pairings are not viable. Tree swallow (*Tachycineta bicolour*) nests that had extra-pair young present had higher hatching success than those that were comprised solely of within-pair young (Kempenaers et al. 1999). The authors argue that this is likely due to trading up for genetic compatibility rather than good genes because extra-pair males did not differ from social mates in any number of traits measured, and males that gained extra-pair paternity were equally likely to lose paternity within their own nests. From another angle, a study of great reed warblers (*Acrocephalus* arundinaceus) demonstrated that mates that shared fewer bands fledged more offspring and that this trend was mediated by a greater proportion of eggs hatching (Bensch et al. 1994). Based on pedigree data, matings were not between close relatives, demonstrating the fitness costs that can be incurred even without present inbreeding (Bensch et al. 1994). Combined, the work of Kempenaers et al. (1999) and of Bensch et al. (1994) shows that genetic similarity may be filtering the population such that the nestlings we study are already the result of more compatible pairings.

Due to low instances of extra-pair mating in my song sparrow population, I was unable to compare fitness in extra-pair versus within-pair young. Consequently, my study primarily focuses on how similarity could affect mating interactions. Other studies, however, have demonstrated how extra-pair mate choice for more dissimilar mates could provide benefits to offspring (Johnsen et al. 2000; Garvin et al. 2006; Fossøy et al. 2007; Forsman et al. 2008). Research in our population of song sparrows has shown that offspring of genetically dissimilar parents grow faster than nestlings of genetically similar parents (Potvin and MacDougall-Shackleton 2009). Investigation of feeding rates however showed that females mated to genetically dissimilar males visited their nestlings significantly more often, and genetically dissimilar males showed a similar, but not statistically significant, trend. This suggests that compatibility effects may be amplified by preferential parental investment (Potvin and MacDougall-Shackleton 2009).

In our outbred, migratory population of eastern song sparrows, pairs appear to form randomly with respect to genetic similarity but trade up when mated to a genetically similar mate. This finding contributes to our understanding of how the interaction between paternal and maternal genomes could influence mate choice and supports the assertion that benefits of genetic diversity may not only apply to small, inbred populations (Amos et al. 2001). Future studies of the frequency of extra-pair copulations, rather than fertilizations, could help distinguish between pre-copulatory and cryptic mate choice, and provide information useful for understanding the mechanism for mate choice for compatibility. Females in our population could be using song or smell as a proxy for assessing similarity (reviewed in Chapter 1). Whether individuals are actively or cryptically choosing genetically dissimilar extra-pair males to avoid inbreeding, produce more heterozygous offspring, or increase compatibility at a specific gene remains to be seen, but the finding provides strong evidence for genetic comaptibilty as a mating strategy.

Genetic similarity to social mate did not predict fledging success for males (Figure 3.4a) or females (Figure 3.4b), nor did it predict female fecundity, as measured by clutch size (Figure 3.5). None of these relationships changed when using absolute relatedness instead of similarity to mate relative to similarity to the population (data not shown). In this case, these measures of reproductive success may not be appropriate for understanding the effects of genetic compatibility for many reasons. Nestling mortality in this population is almost always due to predation (egg predation = 16 nests, predation before day 6 = 8 nests, unknown egg stage failure = 1 nest, unknown nestling failure = 1 nest, successful fledging =16 nests), and occasionally due to the presence of a cowbird (*Molothrus ater*), a nest parasite that can easily outcompete song sparrows for food (6 nests hatched with cowbirds, at least one song sparrow nestling died in each cowbird nest). As song sparrows are cowbird egg acceptors (Strausberger and Ashley 1997), variation in cowbird parasitism should be based on nest construction, territory, and defence factors rather than models of compatibility. The high proportion of nests that did not fledge is unlikely to be due to parental genetic similarity, as it can be almost entirely explained by predators and parasites. Similarly, individual female fecundity is highly repeatable across breeding attempts in song sparrows (Smith 1981) and females may not have control over this aspect of their reproductive success. Finding no relationship between fecundity or fledging success and parental genetic similarity is not surprising if adult song sparrows in our population have little or no control over these fitness variables.

Surprisingly, no significant relationships were found between parental genetic similarity and male or female visits per hour (Figures 3.6 - 3.9) in this population. This was unexpected because previous work on our population has shown that females visit their nestlings significantly more often when paired with a genetically dissimilar mate. Males show a similar, although not statistically significant trend (Potvin and MacDougall-Shackleton 2009). That study included male song complexity as another predictor however, and used only seven microsatellite loci to predict genetic similarity. When including song complexity in my model post-hoc, I found no relationships for male visitation rate. In a model with female corrected relatedness and male song complexity however, genetic dissimilarity was a significant predictor of female visits per hour on day 6, and male song complexity was also associated with increased female visitation rates, although the latter trend was not statistically significant (J. Lapierre and J. Kewin unpublished data). This suggests that the combination of song complexity and compatibility affect parental investment strategies.

Parental investment can be viewed as the final opportunity for mate choice (Neff and Pitcher 2005) as either sex can invest maximally in the offspring of their preferred partner. Since this population has high nestling mortality and low adult overwinter survival, investment in offspring is very costly. The differential allocation hypothesis predicts that females paired with superior mates will invest more in the offspring he has sired (Burley 1986, 1988). Most of these studies have focused on attractiveness as indicated by sexually selected traits (deLope and Møller 1993), but have shown that mate choice continues throughout the breeding season (Møller 1992). In populations where parental genetic compatibility affects offspring fitness, investment in offspring when mated to a less similar mate may be optimal. Offspring of genetically dissimilar parents grew faster in my population (Potvin and MacDougall-Shackleton 2009). The faster growth seems to be mediated at least in part by the increased female visitation rates (Potvin and MacDougall-Shackleton 2009). Nestling condition has been consistently shown to significantly influence survival in song sparrows (Hochachka and Smith 1991) and poor nestling nutrition has carry-over effects later in life in terms of later fledge date, slower growth rates, smaller body size, and size of the song-control nuclei (Searcy et al. 2004; MacDonald et al. 2006). Parental investment can vary between broods and seasons (Hochachka and Smith 1991) suggesting that it is a plastic behaviour rather than a fixed trait. Given the importance of parental care in this species, understanding that an individual optimization model may be more appropriate in this context further demonstrates the complexity of mating strategies.

The observational nature of our study yields some inherent limitations. We were logistically limited to using birds that nested within our study site and we were further limited by the high nestling predation rates which biased our study to include only successful nests. Only first clutches were monitored in this and other seasons (Potvin and MacDougall-Shackleton 2009), and first clutches have higher success than subsequent clutches in many populations (Hochachka 1990). Thus, adults may be investing maximally in their first broods regardless of compatibility or good genes. Future studies would benefit from comparing clutches within a season, especially when the same pairs have multiple clutches. Wetzel and Westneat (2009) challenged the use of shared markers for assessing relatedness or heterozygosity and paternity, demonstrating that it can create a bias in favour of less related or more heterozygous males maintaining paternity. The bias is reduced when using more markers that are highly polymorphic, which was the case in my study. A follow-up analysis argued that biases can occur in both directions but are less likely in exclusion-based paternity assignment than those obtained by likelihood methods (Wang 2010). Despite these limitations, the finding that females mated to more genetically similar males were more likely to have extra-pair young is both significant and relevant to our understanding of how genetic similarity affects mating behaviour.

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Chapter 4: General Conclusions

My thesis examined the relationships between individual genetic diversity, fitness, and mating behaviour in a wild, presumably outbred population of song sparrows (*Melospiza melodia)*. Despite substantial support for heterozygosity-fitness correlations (HFCs) in animals (Keller and Waller 2002; Kempenaers 2007), and some previous evidence for such patterns in this species and population (e.g. Reid et al. 2007; Potvin and MacDougall-Shackleton 2009), my results were generally against HFCs (Chapter 2). I found no relationship between genetic diversity and immunocompetence, nestling growth rates, or measures of female reproductive success. This is consistent with the observation that HFCs are generally weak and account for less than 1% of variation in fitness (Chapman et al. 2009). Two notable exceptions, both observed in males, were a positive relationship between standardized heterozygosity (SH) and parental care, and a negative relationship between SH and overwinter survival.

Genetically diverse males visited their nestlings more often (Chapter 2). This finding suggests that highly heterozygous males provide superior care for their offspring. Genetic diversity has been associated with song complexity and body condition in song sparrows (Reid et al. 2005; Pfaff et al. 2007), and has been linked to several other indicators of overall health and condition across taxa. Brown's (1997) theory of mate choice for heterozygosity emphasized that females should choose mates to maximize offspring heterozygosity in most instances, but should prefer heterozygous males (rather than males with whom they would produce heterozygous offspring) in some cases when heterozygous males are better able to provide direct benefits. This may also be the case in the Mandarte Island population of song sparrows, where offspring of inbred fathers were less likely to fledge (Marr et al. 2006). Finally, while heterozygosity is not itself heritable, some population structures produce more heterozygous offspring than expected when females choose heterozygous males (Mitton et al. 1993; Reid et al. 2006; Neff and Pitcher 2008 a, b).

Surprisingly, in 2008 I found that genetically diverse males were less likely to return to breed the following spring, and thus more likely to be categorized as dying overwinter (Chapter 2). A similar trend was found in the following year. This negative relationship between heterozygosity and apparent fitness seemed to be due to transient local genetic effects in both years. Negative local-effect HFCs have been reported in fish (Lieutenant-Gosselin and Bernatchez 2006) and several studies have found annual differences in HFCs that were likely due to environmental changes (Audo and Diehl 1995; David 1998). The negative HFC which I observed in adult survival was likely responsible for another unexpected result uncovered in this population, that nestlings had higher genetic diversity than in adults. Theory predicts that when HFCs exist, they will be stronger in juveniles than adults because inbred juveniles will be selected against early in life, thus leaving more genetically diverse adults (Koehn and Gaffney 1984; David and Jarne 1997; Cohas et al. 2009). Finding the opposite reinforces the hypothesis that the relationship between genetic diversity and fitness, or at least survivorship, may in fact be negative.

Indicators of both survivorship and reproductive success are needed to study fitness comprehensively (Kokko et al. 2002), and thus several proxies for both survivorship and reproductive success were studied. In males, the fact that genetic diversity is positively related to parental care but negatively related to apparent overwinter survival (Chapter 2) may reflect a trade-off between investment in current and future fitness. Such trade-offs between fitness components and timescales are common (reviewed in Chapter 2). With high adult mortality, as in our study population, a terminal investment strategy is thought to be adaptive for many individuals (Ots and Horak 1996). Future studies would benefit from tracking the same males for several breeding seasons, to determine whether those who invest more in the current season (through nest visits, song output and territory defense) are less likely to return to breed in subsequent seasons.

In Chapter 3, I discovered that social mate choice appears random with respect to genetic similarity, but that females related to highly similar males were more likely to produce extra-pair offspring. I propose that social mate choice is primarily aimed at accruing direct benefits to offset the intense predation risks prevalent in this population, whereas extra-pair mating tactics may be aimed at accruing indirect benefits such as genetic compatibility. Mating systems in which females copulate with multiple males often illustrate the importance of indirect benefits (reviewed in Neff and Pitcher 2005). In birds, mate choice for genetic compatibility is generally most apparent when investigating extra-pair mating behaviour (Mays et al. 2008). This study contributes to such findings, and demonstrates the importance of distinguishing between social and genetic mating behaviour. Genetic similarity between socially mated individuals did not predict reproductive success or parental investment (although see Potvin and MacDougall-Shackleton 2009). Although it is possible that pairwise genetic similarity has no effect on reproductive success and parental investment, this apparent lack of relationship could alternatively be due to high nestling depredation that is largely beyond parental control, maximal investment in first broods (Hochachka 1990), or an overly simplified mate choice model. Potvin and MacDougall-Shackleton (2009) included male song complexity as well as genetic relatedness as predictors of parental investment and saw that both predicted female investment. Although examining song complexity was outside of the scope of my thesis research, future multi-season studies may find support for hierarchical or individual optimization mate choice models that include genetic compatibility (Freeman-Gallant et al. 2003; Neff and Pitcher 2005).

Surprisingly, in this study it seems that mate choice for genetic compatibility is occurring despite the findings that more genetically diverse individuals do not fare any better in most components of fitness, and actually have reduced survivorship. This population could have evolved from a population where inbreeding was more prevalent and inbreeding avoidance more adaptive, or the benefits of genetic diversity could be expressed in other environmental contexts, such as changing, stressful conditions. Alternatively, since most extra-pair males were not identified in this study, females could be trading up for an unknown trait.

My study suggests that complex relationships and trade-offs exist between individual genetic diversity and some components of fitness, with more diverse males providing better parental care but perhaps as a result having decreased apparent survival. Genetic compatibility and mating strategies are similarly multi-faceted; whereas social mate choice appears to be independent of genetic similarity, extra-pair mating appears to be influenced by the genetic similarity of one's social mate. Taken together, my results contribute to our understanding of the effects of genetic diversity on fitness and mating behaviour in outbred populations.

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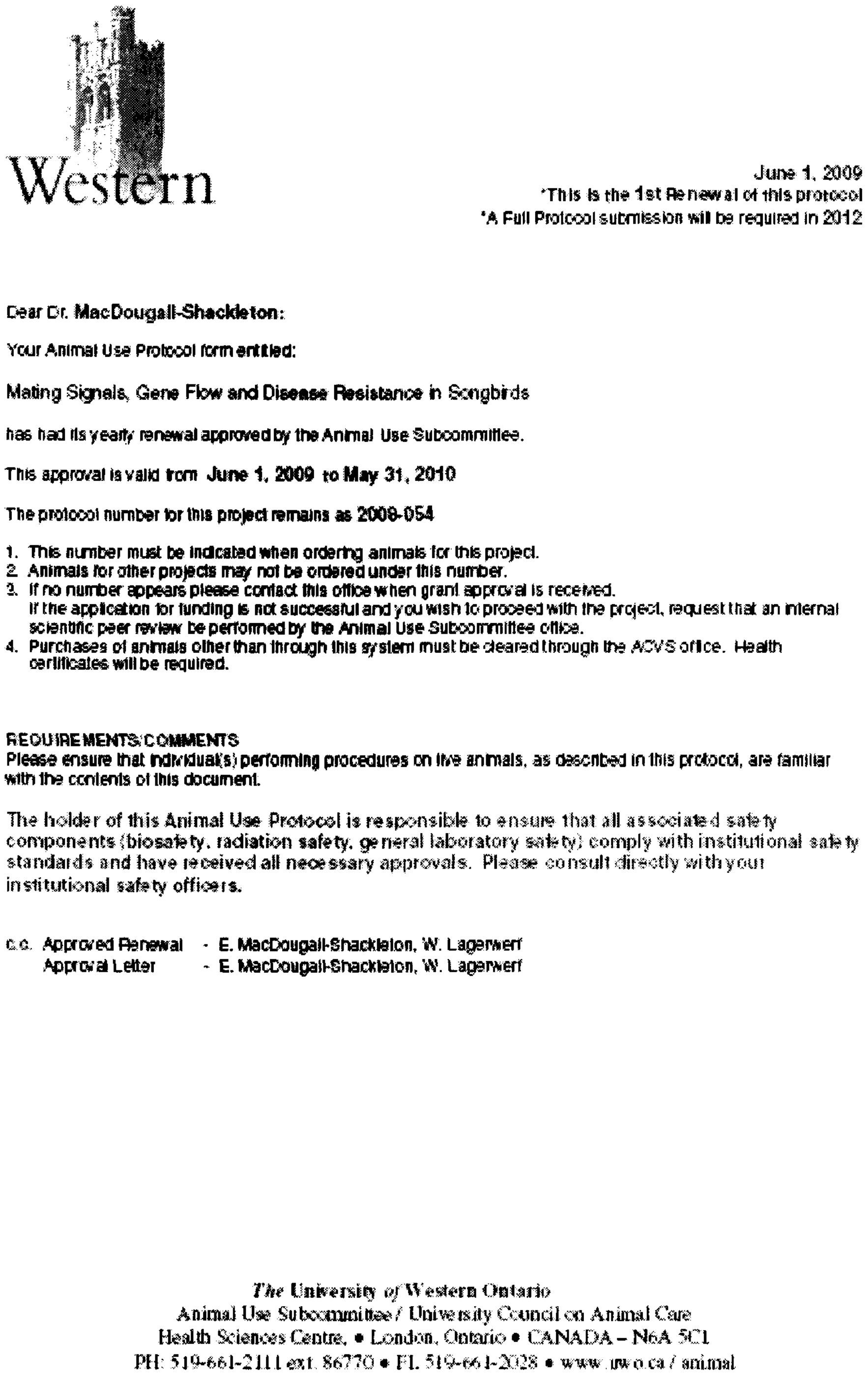
Appendix A: Letter of approval for animal use protocol



Western

June 1, 2009
*This is the 1st Renewal of this protocol
*A Full Protocol submission will be required in 2012

Dear Dr. **MacDougall-Shackleton:**

Your Animal Use Protocol form **entitled:**

Mating Signals, Gene Flow and **Disease Resistance** in Songbirds

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **June 1, 2009** to **May 31, 2010**

The protocol number for this project remains as **2008-054**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

c.c. Approved Renewal - E. MacDougall-Shackleton, W. Lagerwerf
Approval Letter - E. MacDougall-Shackleton, W. Lagerwerf

The University *of* Western Ontario
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