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# PATERNAL ATTRACTIVENESS AND PARENTAL INVESTMENT STRATEGIES IN SONG SPARROWS MELOSPIZA MELODIA

Dominique A. Potvin
*Western University*

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# PATERNAL ATTRACTIVENESS AND PARENTAL INVESTMENT STRATEGIES IN SONG SPARROWS *MELOSPIZA MELODIA*

(Spine Title: Attractiveness and parental investment in song sparrows)

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by

Dominique A. Potvin

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The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

UNIVERSITY OF WESTERN ONTARIO
School of Graduate and Postdoctoral Studies

## CERTIFICATE OF EXAMINATION

Supervisor

Dr. Elizabeth Macdougall-Shackleton

Advisory Committee

Dr. Bryan Neff

Dr. Liana Zanette

Examiners

Dr. Liana Zanette

Dr. Chris Gugliemo

Dr. Greg Thorn

The thesis by

**Dominique Anne Potvin**

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Date ____________________

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## Abstract and Keywords

In many species, both females and males may adjust mating and parental investment tactics in response to the perceived attractiveness of their social mate. I examined how mate attractiveness (based on song complexity and genetic dissimilarity) affects female mating tactics, offspring sex allocation, offspring growth rates and parental feeding rates in a wild population of song sparrows (*Melospiza melodia melodia*). Male song complexity appeared to affect female mating tactics, as males that had smaller song repertoires lost paternity more frequently than did males retaining full paternity within the offspring of their social mate. Paternal song complexity was also predictive of male-biased offspring sex ratios near the end of the nestling period, although not earlier in the nestling period. Males with complex songs also provisioned offspring at higher rates, and consequently their nestlings grew at faster rates than offspring in nests of less complex singers. The degree to which social mates were genetically dissimilar to one another was positively related to rates of offspring growth and female nest visitation. This study contributes to the growing literature focused on the indirect and direct benefits females may receive by mating with highly ornamented or genetically dissimilar males.

Keywords: attractiveness, direct benefits, genetic compatibility, mating tactics, *Melospiza melodia,* parental investment, sex allocation, song complexity, song sparrows

## Co-authorship

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

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# *Chapter 1:* Introduction

Since Darwin's theory of sexual selection was first proposed in 1859, countless studies have focused on the mechanisms by which individuals choose mates and consequently select for certain traits that have contributed to the evolution of populations and species. In most species, females are the limiting and, therefore, choosy sex (Owens and Thompson 1994). There is a large energetic cost of producing ova relative to sperm, and over their lifetime females therefore possess a lower reproductive potential than males (Owens and Thompson 1994). In order to make mating decisions that will increase their reproductive output, females must judge the quality of potential mates. Male quality is often communicated through male secondary sexual traits or ornaments.

Ornaments can serve as honest indicators of male quality often because they are costly ("handicap principle": Zahavi 1975), however this is not always the case. For example, females prefer bright plumage in house finches (*Carpodacus mexicanus*), and this colouration is a result of a diet high in carotenoids (Hill 1990). Carotenoid intake may incur physiological costs, however it may also indicate a male's ability to forage for these rare pigments (Olsen and Owens 1998). Plumage coloration can therefore act as an honest indicator of diet (and hence either territory quality or foraging ability), as well as condition - both important considerations for female reproductive success. These abilities and traits may be inherited or environmentally driven, and therefore may indicate different types of benefits which females might receive.

Direct benefits, such as parental care, the share of a high quality territory or nuptial gifts are often selected for in species whose offspring require a high level of parental care (Møller and Thornhill 1998). For example, in sand gobies (*Pomatoschistus minutus*), both females and males provide parental care, and females tend to choose males that offer higher parental care rather than larger or more dominant males (Forsgren 1997). This selection for direct benefits also ultimately increases female reproductive success. Direct benefits such as paternal care can be indicated by male ornaments (e.g. Keyser and Hill 2000), can often help the survival and development of young (e.g. Eggert *et al.* 1998), and indeed is often essential (Møller 2000).

Male ornaments may also signal indirect, or genetic benefits. Ornaments often indicate an advantageous heritable trait resulting from specific "good genes" that may increase survival and/or future reproductive success. For instance, in great tits (*Parus major*) the breast plumage (a highly variable and honest indicator in males) is inherited directly from father to offspring and is indicative of offspring viability (Norris 1993).

Indirect benefits may also include the effect of compatible genes on offspring fitness (Neff and Pitcher 2005). If there is an advantage to being heterozygous, then females may tend to choose males that are more genetically dissimilar to themselves in order to obtain genetic benefits. Although there may be many advantages associated with heterozygosity due to dominance (limited expression of recessive deleterious mutations or alleles) or overdominance (greater expression of a variety of beneficial alleles) (Tregenza and Wedell 2000), the most well-established is increased immunocompetence (Acevedo-Whitehouse *et al.* 2003, Carrisan-Lloyd *et al.* 2004, Hawley *et al.* 2005). Females, then, may be choosing males based on their genetic dissimilarity, in order to produce more heterozygous (and hence immunocompetent) offspring.

Most male ornaments do not indicate a level of genetic similarity to a particular female, so females must use other cues to determine the genetic compatibility of a potential mate. Such cues may include olfaction (Penn 2002) and/or geographic variation in courtship signals (Marler 1957).

In response to direct and/or indirect benefits females may be receiving from attractive males, females may further increase their reproductive success by altering their parental investment strategies. In order to understand these strategies, it is important to consider the conditions in which they occur.

It has been shown in many species that individuals can and do alter their parental investment strategies according to their current condition (Trivers and Willard 1973, Clutton-Brock *et al.* 1984), environment (e.g. Hargitai *et al.* 2005), and mate attractiveness (Burley 1986, Kolm 2001), in order to increase their lifetime reproductive success. As mentioned above, one variable that has been shown to affect reproductive and parental investment strategies in both males and females is mate attractiveness. Altering investment in response to this attractiveness may occur in a variety of ways.

Firstly, females may alter their reproductive success in socially monogamous systems by choosing to pursue extra-pair copulations. Mating with a male outside the social pair means that a female is likely obtaining genetic benefits (Møller 2000). By mating with males that are genetically superior, females are obtaining good genes for their young. For example, in blue tits (*Parus caeruleus*), females pursue extra-pair copulations with males that are in better physical condition and have more attractive songs, and these benefits are seen to increase survivability in extra-pair offspring (Kempenaers *et al.* 1997). Females may also choose genetically compatible (dissimilar) mates to mate with outside the social pair, meaning extra-pair young are more heterozygous, and can often obtain advantages (Johnsen *et al.* 2000, Foerster *et al.* 2003).

In species where females may obtain both direct and indirect benefits from males, under certain ecological conditions, females may be able to strategize by mating socially with a male that may provide direct benefits to her and her offspring, while pursuing extra-pair copulations with males that have good genes, or compatible genes (Møller 2000). It should be noted that costs have been identified for females that pursue extra-pair copulations, such as decreased care from the social mate when extra pair young are present in the nest (e.g. Sheldon and Ellegren 1998), and time and energy needed to assess other males (Petrie and Kempenaers 1998). However, the frequency with which this strategy is applied across species is testament to its success in socially monogamous systems: extra-pair paternity may be present in upwards of 80% of all broods in some species (Petrie and Kempenaers 1998).

Second, females may increase their reproductive success if they implement certain investment strategies after copulation but before hatch. Females have been shown to alter investment by laying larger eggs (Uller *et al.* 2005) or larger clutch sizes (Petrie and Williams 1993) in response to male attractiveness. Testosterone in the egg may aid in nestling development (Schwabl 1996), and increased levels of these have been found in eggs laid by females mated to more attractive males (e.g. Gil *et al.* 1999, Gil *et al.* 2004).

The sex allocation theory sheds light on another avenue by which female birds may alter their parental investment pre-hatch. Charnov (1982) proposed that in order to increase reproductive success, individuals should invest in the sex that gives them greater fitness returns. If attractive male secondary sexual traits are heritable and increase the reproductive success of sons significantly more than daughters, it would stand to reason that females should invest more in sons when mated to a more attractive male. It has been proposed that females may be able to alter the primary brood sex ratio (sex ratio at hatch) of their young, although the physiological mechanism is not yet known (Pike and Petrie 2003).

After young have hatched, there are further investment strategies that may be employed by both the mother and the father to optimize reproductive success. Females engage in brooding, an essential activity for nestling survival in many species, and both parents may feed nestlings until independence. Female feeding activity has been shown to correlate with mate attractiveness (e.g. Limbourg *et al.* 2004). Nestling development is essential to later survival and reproductive success

(Hochachka and Smith 1991, Naef-Daenzer *et al.* 2001, Nowicki *et al.* 2002), and therefore feeding behaviour by parents may have significant consequences for condition in adulthood. A good example of this is provided by the developmental stress hypothesis (Nowicki *et al.* 1998, Nowicki *et al.* 2002). The developmental stress hypothesis states that the development of neural structures that regulate song learning may be affected by stress experienced as a nestling. This stress can result from environmental conditions such as food availability, for example, a better-fed nestling may therefore be able to learn more complex song. Learned song is often an important sexual ornament that is used by females to make mating decisions (Searcy and Andersson 1986). This is an example of how parental investment can directly influence the future reproductive success of offspring.

Regulating feeding rates to all offspring is one means of altering parental investment, however parents may also engage in preferential feeding of one offspring sex over another. The sex allocation hypothesis predicts that females mated to attractive males may selectively feed sons more than daughters, resulting in lower growth rates for daughters or sex-biased mortality (Kilner 1998, Fawcett *et al.* 2007).

Parental investment is often costly (Owens and Bennett 1994), so females mated to less attractive males may not invest as highly in their offspring. The benefits of raising young that may not be as reproductively successful may not outweigh the energetic or opportunity cost of providing them with high quality care.

### *Birdsong*

Birdsong is an ornament that can convey a great deal of information (reviewed in Searcy and Nowicki 2005). In particular, a large body of research has demonstrated the importance of song complexity (measured as repertoire size) in advertising male quality (e.g. Mountjoy and Lemon 1991, Lampe and Espmark 1994, Buchanan and Catchpole 1997) and the widespread female preference for complex song in males (e.g. Searcy 1984, Mountjoy and Lemon 1996, Lampe and Saertre 1995). Song complexity is associated with offspring survivorship in great reed warblers (*Acrocephalus arundinaceus;* Hasselquist *et al.* 1996), heterozygosity in song sparrows (*Melospiza melodia*; Reid *et al.* 2005) and is also indicative of early developmental condition (Nowicki *et al.* 2002).

This study focuses on a passerine songbird species (*Melospiza melodia melodia*) that is socially monogamous and has a high level of biparental care (Smith *et al.* 1982). Both the mother and the father care for offspring from hatch until independence, which occurs approximately 30 days after hatching (Hochachka and Smith 1991). Young are completely dependent on this parental care until this time, and the level of investment parents provide can have important carryover effects on the fitness of offspring later in life (Hochachka and Smith 1991, Nowicki *et al.* 1998).

Female song sparrows have been shown to prefer males with more complex song repertoires (Searcy 1984, Reid *et al.* 2004). The reasons behind this preference have been speculated on, but are still not fully clear. It is known that song complexity may indicate heterozygosity in song sparrows, which also is an indication of increased immunocompetence (Reid *et al.* 2005, Pfaff *et al.* 2007). Larger repertoire sizes have also been linked with better body condition in males (Pfaff *et al.* 2007). Although these are all important traits, it is still unclear what direct or indirect benefits females are actually receiving and what more attractive males may be passing on to their offspring.

### *Objectives*

In this thesis I examine three major ways in which parental investment strategies by song sparrows (*Melospiza melodia*) might be shaped by male attractiveness (as measured by song complexity) and/or genetic compatibility. In Chapter Two I examine female mating tactics and evaluate the hypothesis that females paired to less attractive males compensate by producing extra-pair offspring. In Chapter Two I also test the hypothesis that paternal attractiveness is related to offspring sex ratios. In Chapter Three I attempt to identify benefits offspring may receive from attractive males in terms of growth rate, and to determine whether such benefits are primarily direct (through parental feeding rates) or indirect (through genetic quality). By isolating the benefits being conferred by attractive males and/or compatible mates, and monitoring female and male behaviors in the presence of naturally occurring variation in mate attractiveness and compatibility, I will evaluate hypotheses about parental investment strategies and how they may evolve in the wild.

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# *Chapter 2:* Effects of male attractiveness on female mating tactics and offspring sex ratios

## Introduction

In many species, females choose mates based in part on secondary sexual traits. Expression of these traits often varies considerably among individuals, and thus, males vary greatly in their attractiveness to potential mates (Anderssen 1994). Indicator models of sexual selection posit that this attractiveness may signal male quality and/or benefits provided to females and offspring. Benefits may be direct, such as parental effort, nuptial gifts or a high quality territory (Mays and Hill 2004). Alternatively, benefits may be indirect, that is indicating a male's genetic quality that could be passed onto offspring (Mays and Hill 2004). Indirect benefits may include "good genes" or "compatible genes" (Neff and Pitcher 2005). In systems where heterozygosity is positively related to individual fitness (reviewed in Keller and Waller 2002), genetic compatibility between two individuals can be estimated from their degree of genetic dissimilarity.

In socially monogamous mating systems, including most bird species (e.g. Lack 1968) not all females may end up paired with an attractive social mate. However, there are two important ways in which females might respond to variation in the attractiveness of their social mate relative to other males in a stable population. First, females can and do respond to variations in male attractiveness by pursuing extra-pair copulations (e.g. Hasselquist and Sherman 2001). Second, in order to further increase the benefits obtained by mating with attractive males, selection can favour parents overproducing the offspring sex that will best contribute to their fitness (Charnov, 1982). This chapter examines both of these strategies in a population of song sparrows (*Melospiza melodia*).

### *Mating Tactics*

Most bird species are socially monogamous (Lack 1968) but extra-pair mating often occurs (Petrie and Kempenaers 1998). Male ornaments, such as colourful plumage in yellow warblers (*Dendroica petechia;* Yezerinac and Weatherhead 1997), elaborate displays in superb fairy-wrens (*Malurus cyaneus;* Dunn and Cockburn 1999), or song characteristics in great reed warblers (*Acrocephalus arundinaceus;* Hasselquist *et al.* 1996) have been implicated in female mating decisions, both in terms of social and extra-pair mate selection. These characteristics can indicate direct and/or indirect benefits, and females paired with unattractive social mates can increase their likelihood of obtaining these benefits if they engage in extra-pair mating with more attractive males (Hasselquist and Sherman 2001).

Elaborate male ornaments may signal a variety of direct benefits. For instance, male red-winged blackbirds (*Agelaius phoeniceus*) use song to advertise higher quality territories (Yasukawa *et al.* 1980); sedge warbler (*Acrocephalus schoenobaenus*) song advertises higher offspring feeding rates (Buchanan and Catchpole 2000); and bright coloration in male blue grosbeaks (*Guiraca caerulea*) has been shown to correlate with both territory and parental quality (Keyser and Hill 2000. Females may also choose males with better genes that contribute to offspring survival or condition. For example, female blue tits (*Parus caeruleus*) choose extra-pair mates that sing longer strophes and have longer tarsi (both indicators of genetic quality) than their social mates, and the extra-pair offspring from these pairings survive at higher rates (Kempenaers *et al.* 1997). Females may also choose males that are very genetically dissimilar to themselves, as seen in Savannah sparrows (*Passerculus sandwichensis;* Freeman-Gallant *et al.* 2006), in order to produce more heterozygous offspring, in light of the positive relationships often observed between heterozygosity and fitness (e.g. Foerster *et al.* 2003, reviewed by Hansson and Westerberg 2002).

In song sparrows, females have been shown to prefer males with more complex song repertoires (Searcy 1984, Nowicki *et al.* 2002, Yasukawa 2002, Reid *et al.* 2004). Adult song complexity has been shown to indicate genetic quality (reviewed by Catchpole 1996 and Scharff and White 2004) in many species, including great reed warblers (Hasselquist *et al.* 1996), as well as song sparrows (Searcy *et al.* 1985), where fitness benefits are correlated with song complexity (Reid *et al.* 2005a). Male song complexity may also indicate increased paternal effort, as in sedge warblers (Buchanan and Catchpole 2000). In song sparrows, song complexity is related to both individual genetic diversity and immunocompetence (Reid *et al.* 2005b, Pfaff *et al.* 2007). As a result, females choosing males with higher song complexity as their social and/or genetic mating partners may obtain direct and/or indirect benefits for their offspring, including increased nutrition, decreased susceptibility to parasites and increased reproductive success (Reid *et al.* 2005a).

In addition to obtaining indirect benefits by choosing a male with good genes, females may also enhance offspring fitness by choosing a male with compatible genes (Neff and Pitcher 2005). Specifically, mated pairs who are relatively dissimilar genetically should tend to produce more heterozygous offspring relative to more genetically similar mated pairs (e.g. Tarvin *et al.* 2005). It is important to note that these two theories of mate choice (good genes vs. compatible genes) are not mutually exclusive; rather both can operate in the same population (Mays and Hill 2004). In song sparrows, song complexity may indicate good genes and/or heterozygosity (Reid *et al.* 2005a, Reid *et al.* 2005b, Pfaff *et al.* 2007). Genetic compatibility may be assessed through other cues, such as olfaction (Zelano and Edwards 2002).

In many avian species, females appear to enhance offspring fitness through extra-pair mating. Although in some cases females may obtain direct benefits from extra-pair mating (e.g. red-winged blackbirds *Agelaius phoniceus;* Gray 1997), more often the benefits associated with extra-pair mating are indirect. A growing body of evidence suggests that females may obtain good genes for their offspring from extra-pair mates (e.g. blue tits Kempenaers *et al.* 1992, great reed warblers Hasselquist *et al.* 1996, Black-capped chickadees *Parus atricapillus* Otter *et al.* 1998, also see review by Hasselquist and Sherman 2001). Females can also increase offspring heterozygosity through extra-pair copulations in blue tits (Foerster *et al.* 2003) and splendid fairy-wrens (*Malurus splendens;* Tarvin *et al.* 2005). The greater heterozygosity of extra-pair relative to within-pair offspring may help to explain the greater immunocompetence of extra-pair offspring as in bluethroats (*Luscinia svecica;* Johnsen *et al.* 2000).

My objective in this section of my thesis was to examine how variation in male attractiveness (as determined by song complexity and genetic dissimilarity between social mates) affects female mating tactics. If female song sparrows prefer males with more complex song, then males with smaller repertoire sizes should be more likely to lose paternity than males with larger repertoire sizes. If females are choosing mates based on genetic compatibility (specifically, genetic dissimilarity) then males that are highly genetically dissimilar to their social mates should be more likely to retain paternity in the offspring of their social mate, relative to males that are genetically similar to their social mate. Moreover, if female song sparrows pursue extra-pair mating primarily with genetically compatible (dissimilar) males, extra-pair offspring may be more heterozygous than within-pair offspring.

### *Sex allocation*

Another major route through which parents may enhance their own fitness is to adjust the sexes of offspring they produce in response to paternal attractiveness. Parental investment theory predicts that parents should invest more in the offspring sex that will give them greater fitness returns (Charnov 1982). At the population level, this theory generally results in a sex ratio close to unity (Fisher 1930). However, this ratio may not hold at an individual level, because of constraints and variation in quality of individual parents (Trivers and Willard 1973). Charnov (1982) explained this individual variability through his classic theory of sex allocation, wherein a bias in offspring sex allocation would be adaptive if the gain in fitness through one sex exceeded the loss through the other sex. Originally applied to haplo-diploid insects, this sex allocation theory has since been successfully applied to mammals and birds (Clutton-Brock *et al.* 1984, Komdeur and Pen 2002).

Although the exact mechanisms of sex allocation in birds have not been determined, many theories have been put forward (reviewed in Pike and Petrie 2003). For example, selective feeding of sons over daughters (or vice versa) is one method of altering the secondary brood sex ratio (i.e. the ratio of sons to daughters at the end of parental care). However, it may be more feasible and less costly to bias the primary brood sex ratio (i.e. the ratio of sons to daughters at fertilization). One potential mechanism by which songbirds might adaptively adjust the primary sex ratio of their offspring is selective ovulation, whereby ova with the unwanted sex chromosome are shed following meiosis (Pike and Petrie 2003).

There are many selective conditions that have been suggested to favour offspring sex allocation bias at the individual level. Trivers and Willard (1973) proposed that parental quality, specifically maternal condition, was a major consideration in adaptive sex ratio bias. The "sexy son" hypothesis (Weatherhead and Robertson 1979) originally postulated to explain female tolerance of polygyny also predicts that females mated to elaborately ornamented (attractive) males should produce predominantly male offspring. This hypothesis assumes that sons of an attractive male benefit more from inheriting an attractive trait than do daughters inheriting the same genes. Supporting evidence comes from studies on several bird species, including blue tits (Dreiss *et al.* 2006), canaries (*Serinus canaria;* Leitner *et al.* 2006), collared flycatchers (*Ficedula albicollis;* Ellegren *et al.* 1996) and zebra finches (*Taeniopygia guttata;* Burley 1981, 1986). However, other studies have found no correlation between paternal attractiveness and offspring sex ratio, for example in great reed warblers (Westerdahl *et al.* 1997), black-capped chickadees (Ramsay *et al.* 2000), and dark-eyed juncos (*Junco hyemalis;* Grindstaff *et al.* 2004). These mixed results may indicate a weak selective advantage to manipulating offspring sex ratios and/or mechanistic constraints in certain species (Westerdahl *et al.* 1997, Komdeur and Pen 2002, Fawcett *et al.* 2007).

In song sparrows, the advantages that sons receive from fathers with more complex song are not completely understood. Although the degree to which song complexity is heritable has not yet been established, Airey *et al.* (2000) have demonstrated a heritable component to song control nuclei size in zebra finches. There may be other benefits, both indirect (Hasselquist *et al.* 1996) and direct (Buchanan and Catchpole 2000) associated with song complexity that may affect the success of sons more than daughters. It should be noted that the effects of behaviours and traits/ornaments that are solely *learned* on offspring sex ratios remains an open question.

In our study population of song sparrows, as of yet, heterozygote advantage has been observed only in males through the expression of secondary sexual traits (Pfaff *et al.* 2007) and apparent resistance to haematozoan parasites (Singh 2007). This raises the possibility that sons may benefit more than daughters from parental genetic compatibility. Thus the "outbred son" hypothesis predicts that proportion of sons should be negatively related to genetic similarity between members of a mated pair.

My objective in this section of my thesis was to determine whether male attractiveness (as determined by song complexity and genetic dissimilarity to social partner) is associated with male-biased primary and/or secondary offspring sex ratios. The "sexy son" hypothesis predicts that males that are more attractive should produce male-biased offspring sex ratios. The "outbred son" hypothesis predicts that mated pairs that are genetically dissimilar should also produce male-biased offspring sex ratios.

## Methods

### *Study site and study population*

This study was conducted from 2007 to 2008 on land owned by the Queen's University Biology Station, near Newboro, Ontario, Canada (44° 38' 60 N, 76° 19' 0 W). I also had access to data (recordings and familial DNA) from this population and study site from 2006, and included these in my analyses. The study population consists of approximately 25 to 40 breeding pairs of song sparrows (*Melospiza melodia melodia*). This population is socially monogamous, with a moderate level of extra-pair paternity (5-20% of broods). As in other populations of song sparrow, repertoire size of our study population ranges from 5-13 song types per male.

Previous work on this population has shown that repertoire size is associated with male body condition and the size of song nuclei (HVC) in the brain (Pfaff *et al.* 2007).

### *Field methods*

All adult song sparrows were captured in mist nets or seed-baited treadle traps in April and May of 2006, 2007 and 2008. Any birds without bands were issued a uniquely numbered Canadian Wildlife Service leg band along with a unique combination of three colour bands to permit individual identification in the field. A small (<25μL) blood sample was collected from the brachial vein and blotted onto Whatman 113 high wet-strength filter paper for subsequent genetic analysis (see below).

### *Song analysis*

Each male's song repertoire was recorded with Marantz Professional PMD 671 solid state recorders and Telinga Twin Science Pro parabolic microphones. We considered a sample of a male's repertoire (the total number of song types) to be complete after recording either 300 consecutive songs or 450 non-consecutive songs, following Searcy *et al.* (1985) and Cassidy (1993). Returning males whose repertoires had already been recorded in previous field seasons were not re-recorded, as song sparrows are closed-ended learners and song repertoires do not change in adulthood (Nordby *et al.* 2002).

I used the computer software SYRINX v.2.6h (John Burt; www.syrinxpc.com) to generate spectrograms of all recorded songs. To quantify song complexity I classified each male's complete set of recordings into distinct song types by visual analysis and sorting following Pfaff *et al.* (2007).

### *Nest location and monitoring*

Members of the research team located nests by behavioral observations, and monitored them until nestlings hatched. On the second day after hatch, I collected small blood samples (<25μL) from the tarsal vein of each nestling and I measured mass to the nearest 0.1g using a spring scale. Nestlings' toes were marked using a felt tip marker to identify individuals so I could track survival and growth to day six. I collected unhatched eggs at this time for subsequent genetic analysis of embryos (if detectable). I then banded nestlings on the sixth day after hatch, and mass measurements were retaken using a spring scale to the nearest 0.5g, in order to estimate growth rates in grams per day.

### *Microsatellite analysis*

I extracted DNA from all individuals' blood samples using an ammonium acetate-based protocol to salt out proteins, modified from Laitinen *et al.* (1994). The extracted DNA was then made into a working dilution (with distilled water) that could be used in subsequent polymerase chain reactions (PCR).

All birds (adults and nestlings) in the population were genotyped at seven microsatellite loci: Mme2, Mme7 (a sex-linked microsatellite) (Jeffrey *et al.* 2001), Escμ1 (Hanotte *et al.* 1994), Pdou5 (Griffith *et al.* 1999), SOSP3, SOSP13, and SOSP14 (Lukas Keller, pers. comm. to Dr. MacDougall-Shackleton). One primer at each locus was dye-labeled (Integrated DNA Technologies) and microsatellites were amplified using polymerase chain reaction. PCR was conducted in a total volume of 10 μL and included 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 0.2 mg/mL BSA, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1-0.4 mM of each primer, 0.5 U *Taq* polymerase (Fisher Scientific) and approximately 25 ng of genomic DNA. Cycling conditions included an initial step of 60 s at 94°C, followed by 29 cycles of 30 s at 94°C, 30 s at the annealing temperature (90 s for Sosp 3, 13 and 14), and 60 s at 72°C, plus a final step of 270 s at 72°C. Annealing temperatures were 54°C for Pdou 5 and 57°C for Sosp 3, 13 and 14. Escμ 1, Mme 2 and Mme 7 were amplified in a touchdown reaction with annealing temperatures dropping from 58°C to 53°C. The resultant PCR products were analyzed on a Beckman-Coulter CEQ 8000 (2006 and 2007) or an Applied Biosystems 3130 Genetic Analyzer (2008). I tested for deviations from Hardy-Weinberg expectations and from linkage equilibrium using GENEPOP version 3.3 (Raymond and Rousset 1995) and found no evidence for either.

I used microsatellite profiles to calculate Wang's (2002) coefficient of relatedness using the software MARK (Kermit Ritland, http://genetics.forestry.ubc.ca/ritland/programs.html). I also used these profiles to identify extra-pair offspring and paternity. If nestlings' genotypes did not match with those of their social father at one or more loci, but were compatible with those of social mothers, then these were confirmed as extra-pair offspring. I observed no cases of nestlings whose genotypes were incompatible with those of their social mothers. To assign paternity, I conducted a search to match the paternal loci from extra-pair offspring to a male within the population: in order to confirm paternity, all loci not from the mother had to be compatible with those of the father.

I estimated nestling homozygosity using HL (homozygosity by loci; Aparicio *et al.* 2006) as calculated by the IRMacroN4 (William Amos; http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms). This estimate takes into account the allelic variability of each locus included in the analysis and subsequently weights each locus according to this variability. HL is considered a reliable index for an individual's genetic diversity within a population (Aparicio *et al.* 2006).

### *Sex determination*

I determined the sex of 222 nestlings and unhatched eggs from 64 nests over the three years. I used the primers P2 and P8 (Griffiths *et al.* 1998) to amplify portions of the CHD-W and CHD-Z gene (found on avian sex chromosomes) following Griffiths *et al.* (1998). PCR was conducted in a total volume of 15 μL and included 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100, 0.2 mg/mL BSA, 3.9 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM each of P2 and P8, 0.375 U *Taq* polymerase (Fisher Scientific) and approximately 75 ng of genomic DNA. I separated PCR products by electrophoresis at 60-80 V for 60-90 minutes on 1.5% agarose gels stained with ethidium bromide, then visualized PCR products under ultraviolet light. In birds, females are the heterogametic sex, thus samples amplified from females show two bands (CHD-Z, CHD-W) and samples from males only one (CHD-Z). I took digital photographs of each gel and included control amplifications from a known male and a known female.

### *Statistical Analysis*

All statistical tests were two-tailed and all data were normally distributed. I used an unpaired t-test to compare the repertoire sizes of males that had lost paternity in the nest of their social mate, to those of males with complete paternity within their social mate's nest. I also used t-tests to compare the homozygosity of extra-pair versus within-pair offspring for all three years.

I investigated the relationship between offspring sex ratios and paternal attractiveness (repertoire size and pairwise relatedness) using a generalized linear model regression with a logit link function and binomial error distribution (PROC GENMOD in SAS 9.1). I constructed two models to test the hypothesis that song complexity and/or genetic dissimilarity of mates correlated with brood sex ratio. For the first, I included all variables of interest: year, standardized hatch date, number of offspring, number of sons, father song repertoire size and coefficient of relatedness of parents. I then used backward elimination of possible explanatory variables and their interactions to create a simpler model with acceptable explanatory power. For each nest, number of sons was the response variable and total number of offspring was the binomial denominator. Embryos collected from unhatched eggs were included (along with hatched nestlings) in the day 2 sex ratio analyses, while day 6 analyses included only surviving nestlings. I carried out a post-hoc power analysis for the non-significant results following the procedures in the freeware program G*Power (Faul and Erdfelder 1992).

## Results

### *Mating tactics*

64 nests containing a total of 222 individual nestlings were analyzed. Mean brood size was 3.47 (range 1 - 5; SE = 0.145), Extra-pair paternity occurred in 18.7% of nests (13/64), and overall, 8.6% of nestlings (19/222) were extra-pair.

Males that lost paternity within the offspring of their social mate had smaller repertoire sizes on average than males that retained full paternity in their nest (t = -2.35, df = 55, P = 0.023; Figure 2.1). Because full song repertoires were not available for all of the males in the population, low sample size for this part of the analysis precluded me from comparing repertoire sizes of extra-pair versus social (cuckolded) fathers. Variation in sample size for the following analyses reflects the fact that repertoires were recorded for most, but not all adult males in the study.

Males that lost paternity within the offspring of their social mate were not more or less genetically similar to their social mates than were males with full paternity (t = -0.43, df = 59, P = 0.966). Similarly, females that produced extra-pair offspring were not more or less related to their extra pair mates than to their social mates (t = -1.47, df = 17, P = 0.159). However, extra-pair young were significantly less homozygous than within-pair young (t = -2.33, df = 203, P = 0.021, Figure 2.2).

Table 2.1 – Descriptive statistics of biological data collected from song sparrow population 2006-2008

| | **Mean ± S.D. (n)** | **Range** |
|---|---|---|
| Male song repertoire size | 8.2 ± 1.8 (57) | 5.0 – 12.0 |
| Relatedness (Wang 2002) between social mates | -0.015 ± 0.067 (32) | -0.124 – 0.109 |
| Nestling homozygosity by loci (HL) | 0.189 ± 0.134 (222) | 0.00 – 0.57 |
| Julian hatch date | 149.4 ± 8.9 (59) | 135 - 167 |

### *Sex allocation*

The total sex ratio (proportion male) of all nestlings on day 2 was 0.51 (113/222), and on day 6 was 0.56 (75/135) consistent with the population sex ratio predicted by Fisher (1930). I found a significant difference between years for day 2 sex ratios ($\chi^2$= 6.01, P = 0.014 Figure 2.3a), however I found no difference in day 6 sex ratios between years ($\chi^2$= 1.34, P = 0.245 Figure 2.3b). Accordingly, I included year as a variable in the tests reported below.

Neither day 2 nor day 6 brood sex ratios were found to correlate with mate attractiveness in this model. Offspring sex ratio on day 2 was not correlated with social father's repertoire size ($\chi^2$= 0.34, P = 0.56), nor was it correlated with parental relatedness ($\chi^2$ = 0.25, P = 0.62, Figure 2.4a). The power of this test was 78% for a moderate effect size. Day 6 sex ratios also showed no significant relationship (although borderline) with social father's repertoire size ($\chi^2$ = 3.28, P = 0.07), or parental relatedness and brood sex ratio ($\chi^2$ = 0.77, P = 0.38, Figure 2.4b). Sex ratios were also unrelated to hatch date (day 2: $\chi^2$ = 0.13, P = 0.72; day 6: $\chi^2$ = 0.75 P = 0.39).

Following other studies (Ewen *et al.* 2002, Rutstein *et al.* 2005) I re-ran a second reduced model that included only year and social father's repertoire size as predictor variables. Under this model, although sex ratio on day 2 remained unrelated to paternal repertoire size ($\chi^2$ = 0.52, P = 0.47, Figure 2.5a), sex ratio on day 6 was positively related to male repertoire size ($\chi^2$ = 4.66, P = 0.031, Figure 2.5b).

To explain alteration in sex ratios at day 6, one of two possibilities were likely occurring: either there was significant mortality of female nestlings in attractive males' nests (creating a male bias), or there was significant mortality of male nestlings in less attractive males' nests (creating a female bias). I ran a t-test to investigate the differences between the social fathers' repertoire sizes for nests in which male and female nestlings were dying. I found that daughters were consistently dying in nests of males with larger repertoire sizes ($t = -3.78$, $df = 64$ $P = 0.0003$). I then also examined the growth rate between brothers and sisters in the same nest, which may help to explain the differences in mortality, and found that brothers consistently grew faster than sisters ($t = -2.88$, $df = 20$, $P = 0.009$).

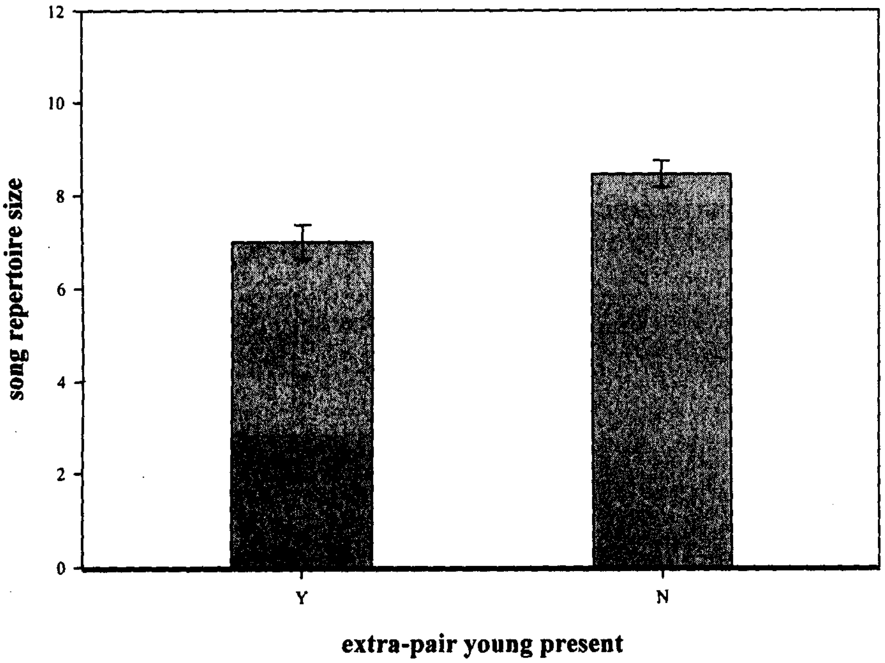


Figure 2.1 – Mean (±SE) repertoire size of males that lost paternity within the nest of their social mate, versus males that retained full paternity. Males that lost paternity had smaller repertoire sizes than males with full paternity.

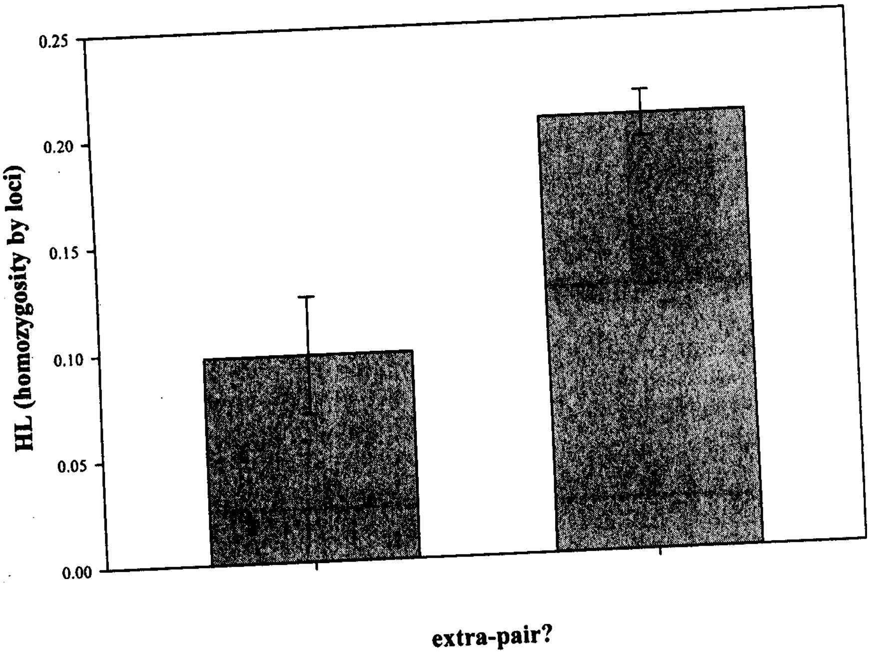


Figure 2.2 – Homozygosity by loci (HL: Aparicio *et al.* 2006) of extra-pair and within-pair offspring. Offspring resulting from extra-pair copulations were less homozygous than those sired by the male at the nest.

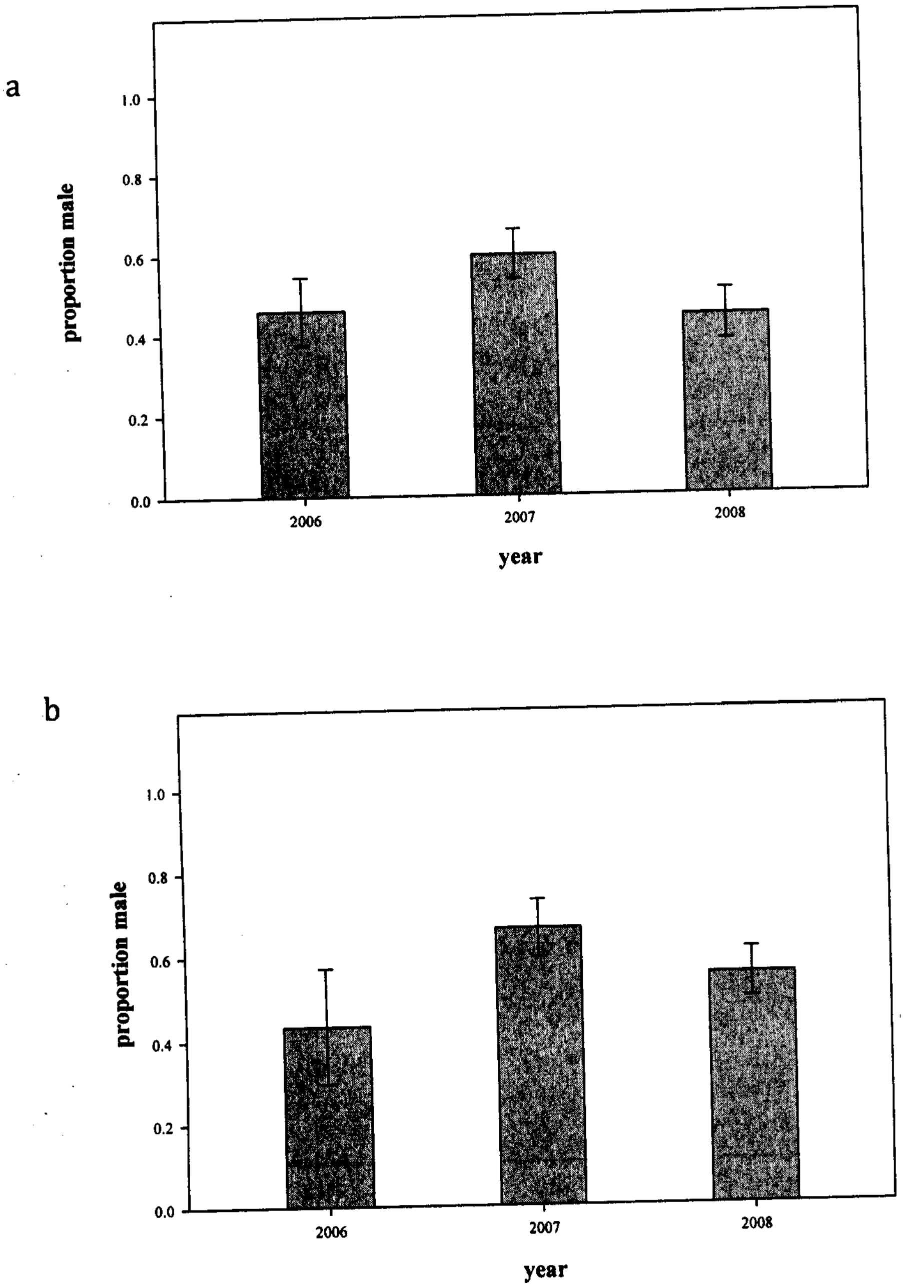
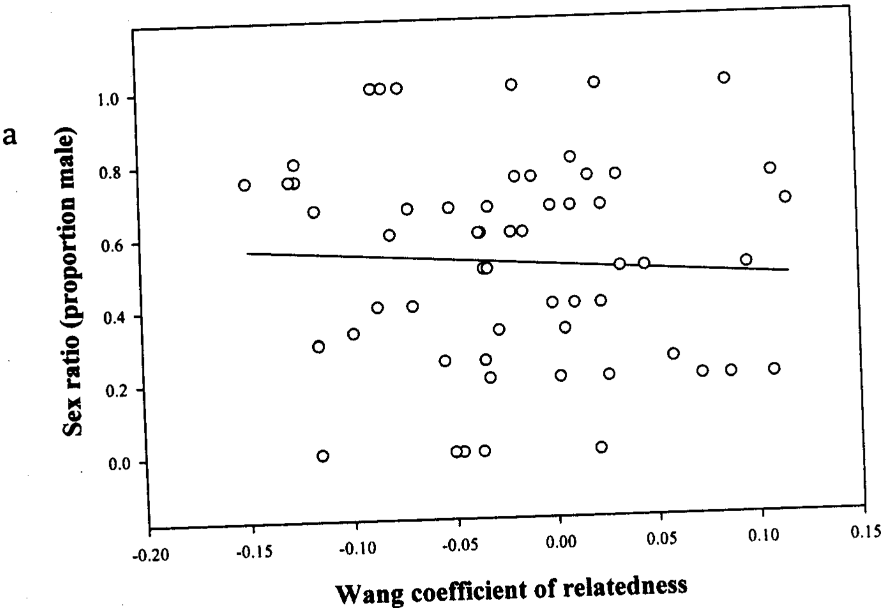


Figure 2.3 – Year effects on a) day 2 and b) day 6 sex ratios. Primary, but not secondary, offspring sex ratios were significantly more male-biased in 2007. Sex ratio is represented as mean (±SE) proportion of sons in the nests.



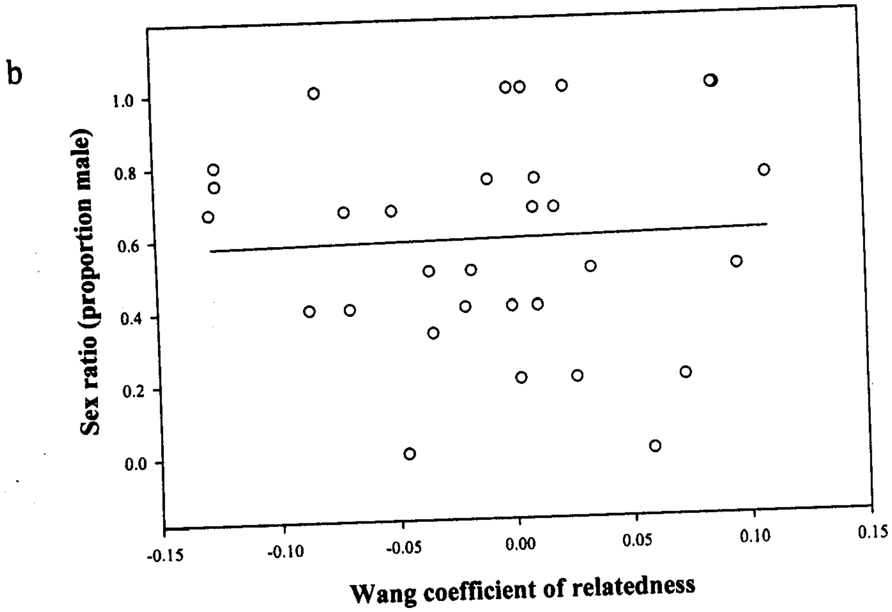


Figure 2.4 – Relationship between parental relatedness (Wang 2002) and offspring sex ratio on a) day 2 and b) day 6. A least-squares regression is plotted for purposes of illustration.

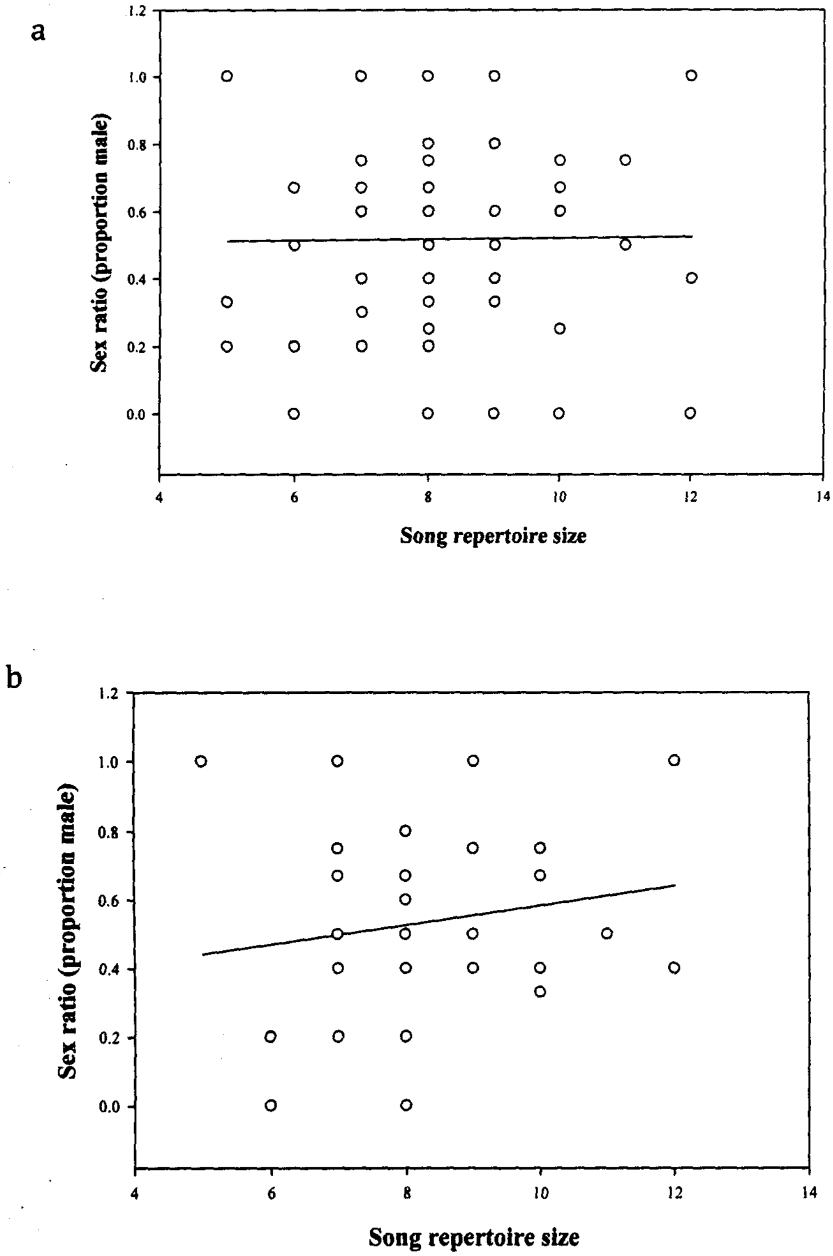


Figure 2.5 - Relationship between paternal repertoire size and offspring sex ratio on a) day 2 and b) day 6. Sex ratio is represented as proportion of sons. A least-squares regression is plotted for purposes of illustration.

## Discussion

My findings support the idea that females are adjusting their mating tactics in response to their social partner's repertoire size. As well, the finding that extra-pair offspring are more genetically diverse than within-pair offspring suggests that genetic compatibility may also affect female mating decisions. Finally, this study presents some evidence that offspring sex ratios are related to paternal attractiveness in this species, although interestingly, this appears to be mediated through post-hatch differences in survival rather than through facultative adjustment of primary sex ratios. Collectively, these findings suggest that 1) song sparrow females are adjusting mating tactics based on the attractiveness of their social mate; and 2) song sparrow nestling mortality may contribute to a secondary sex ratio bias.

### *Mating tactics*

Although the sample size for this study was too small to directly compare the repertoire sizes of males losing paternity through extra-pair copulations to those gaining paternity through extra-pair copulations, I did uncover evidence supporting the idea that male repertoire size affects female mating tactics. Males with smaller song repertoires were much more likely to be cuckolded than were males with larger repertoires. This pattern is consistent with findings by Hasselquist *et al.* (1996) in great reed warblers, by Houtman (1992) in zebra finches as well as others (blue tits Kempenaers *et al.* 1992, black-capped chickadees Otter *et al.* 1998, dusky warblers (*Phylloscopus fuscatus*) Forstmeier *et al.* 2002). To the extent that song learning ability advertises a male's good genes (Catchpole 1996, Scharff and White 2004) females may be adjusting their mating strategies based on their social mate's repertoire size in order to obtain heritable benefits for their offspring.

My findings contribute to a growing body of evidence that females may select extra-pair mating partners based at least in part on genetic compatibility. I observed that extra-pair young were significantly less homozygous than within-pair young (Figure 2.2). Foerster *et al.* (2003) found that blue tits also increase the heterozygosity of their offspring through extra-pair mating, and Tarvin *et al.* (2005) found the same effect in the splendid fairy-wren. In song sparrows, where male heterozygosity is associated with repertoire size (Reid *et al.* 2005a, Pfaff *et al.* 2007), and increased immunocompetence (Reid *et al.* 2005a, Singh 2007) the advantages of producing more heterozygous young are apparent. Although the level of extra-pair paternity in this system is relatively low, females do appear to adjust their mating tactics in order to obtain good and/or compatible genes for their offspring.

### *Sex Allocation*

Consistent with the predictions of the 'sexy son hypothesis', offspring sex ratios near the end of the nestling period were significantly related to paternal repertoire size. Surprisingly, however, this effect did not appear to be mediated through facultative manipulation of primary sex ratios, because repertoire size was not related to sex ratios earlier in the nestling period (Figure 2.5a). Although some taxa appear to adjust primary offspring sex ratios according to mate attractiveness (e.g. zebra finches Burley 1986, collared flycatchers Ellegren *et al.* 1996, and blue tits

Dreiss *et al.* 2006), others do not (great reed warblers Westerdahl *et al.* 1997, barn swallows *Hirundo rustica* Saino *et al.* 1999 and canaries Leitner *et al.* 2006). Altering primary sex ratio is presumably less costly than adjusting sex ratio post hatch via differential mortality, so the absence of evidence for primary sex ratio manipulation may reflect the birds' inability to do so. Pike and Petrie (2003) reviewed a number of proposed mechanisms through which birds might manipulate primary sex ratios, however no study has yet demonstrated the existence of any of these in song sparrows.

The correlation between secondary sex ratio and repertoire size in this population may support the sex allocation hypothesis if the reason for the sex ratio bias is differential feeding between male and female offspring. I did observe a difference in growth rates between brother and sister nestlings. This suggests that parents may be engaging in differential feeding of one sex over the other. However, it is unlikely that adult birds can assess the sex of individual nestlings while feeding, or that adults do not treat male and female nestlings differently (e.g. Lessells *et al.* 1998), and therefore it is unlikely that sex allocation is occurring.

The finding that sons grow faster than daughters also indicates that sons may be more costly to raise, a trend observed in other species (Bradbury and Blakey 1998, Nowicki and Searcy 2004). Males that are better quality (as indicated by their repertoire size) may be able to feed these offspring more, helping male nestling survival in these nests. There is overwhelming evidence in many species that more attractive males are better providers (e.g. zebra finches; Burley 1986, house sparrows (*Passer domesticus)* Voltura *et al.* 2002 and sedge warblers; Buchanan and Catchpole 2000). The secondary sex ratio bias could therefore be a product of how male attractiveness affects parental ability or effort. However, when mortality was compared between female and male nestlings, it was found that the day 6 sex ratio bias was a result of female deaths in nests of more attractive males. The reason for these deaths is not completely clear. One possibility is that male nestlings that are fed more often, perhaps by an attractive male, may be better able to outcompete daughters. There may be a positive feedback effect where the larger a male nestling grows, the better able he is to beg for more food, the more food he receives and the larger he grows, etc. Because male nestlings grow faster than their female siblings, this effect would have a negative impact on the ability of females in the nest to receive food. It has been found that cowbird nestlings in song sparrow host nests create a similar effect: female song sparrows in parasitized nests are susceptible to being outcompeted (and dying) by the cowbird (a fast grower and excellent beggar) (Zanette et al 2005).

Contrary to the expectations of the "outbred son" hypothesis, I observed no relationship between parental genetic similarity and offspring sex ratios (either shortly after hatch or closer to the end of the nestling period). This might imply that females cannot easily determine genetic similarity of their social mate. However, this is unlikely as I also found extra-pair offspring to be more genetically diverse than within-pair offspring. Alternatively, these results may suggest that heterozygote advantage is not necessarily stronger in males than in females. Studies have shown heterozygote advantages in males, for instance more heterozygous males have higher immunocompetence and larger song repertoire sizes (Reid *et al.* 2005b, Pfaff *et al.* 2007). However, other studies have also found female heterozygote advantage to females in terms of fecundity (Ortego *et al.* 2007) and hatching success (Marr *et al.* 2006). If the relationship between individual genetic diversity and fitness is similar in both females and males, then there may be no real selective advantage to manipulating brood sex ratio in response to expected offspring heterozygosity.

### *General Conclusion*

As with any field study, there are limitations to testing natural correlations and other unmeasured variables may also be implicated in female mating tactics and offspring sex ratios. There are many other variables that have been shown to influence female mating tactics in other species, such as age and proximity (house sparrows *Passer domesticus* Wetton *et al.* 1995) as well as territory quality (great reed warblers *Acrocephalus arundinaceus* Hasselquist *et al.* 1996). In addition, territory quality (Appleby *et al.* 1997, Komdeur *et al.* 1997, Hasselquist and Kempenaers 2002), weather conditions, laying order and egg size effects (Albrecht 2000) as well as parental stress levels/physiological states (Bonier *et al.* 2007) have all been suggested to affect sex ratios in different avian species. Controlled laboratory studies of mating preferences and offspring sex allocation can provide a powerful way of testing hypotheses about female investment strategies, especially when combined with studies of free-living birds in their natural environment such as this one.

The next chapter discusses the relationship between mate attractiveness and parental investment by both sexes. Because environmental effects on nestlings may have long-lasting effects on adult condition and fitness, it is important to consider investments beyond extra-pair mating and sex allocation as a measure of parental investment in nestlings.

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# *Chapter 3:* The effect of paternal attractiveness and genetic similarity between mates on offspring performance and parental effort

## Introduction:

In many avian mating systems, females make mating choices based in part on male ornamental traits (Andersson 1986). Such ornaments often vary highly in attractiveness between individuals, and may be honest indicators of male quality (Andersson 1986). By choosing an elaborately ornamented mate, females may gain direct and/or indirect benefits for themselves or their offspring. For instance, elaborately ornamented males may provide direct benefits in the form of parental care to offspring through high levels of feeding and/or defense. These direct benefits have been shown to occur in many bird species, for example, brighter plumage in eastern bluebirds (*Sialia sialis*) indicates higher male provisioning rates to offspring (Siefferman and Hill, 2003). A similar relationship was found with male badge size and feeding rates in house sparrows (*Passer domesticus;* Voltura *et al.* 2002) and in northern cardinals (*Cardinalis cardinalis*) Wolfenbarger (1999) found a correlation between plumage colouration and territory quality of males.

Elaborate ornaments may also signal a male's ability to provide indirect benefits or 'genetic' benefits to offspring. Traditionally, indirect benefits have been attributed to "good genes" that may be passed on from father to offspring and confer additive benefits on offspring fitness regardless of maternal genotypes (Møller and Alatalo 1999). For example, in great tits (*Parus major*), the length of plumage stripes is a highly heritable trait that is passed down from father to son and has a direct influence on male reproductive success (Norris 1993).

Recently, our understanding of genetic quality has expanded to consider compatible genes effects on fitness (Brown 1997, Neff and Pitcher 2005), defined as nonadditive interactions between alleles (e.g. heterozygote advantage). Heterozygosity is often positively related to various aspects of fitness (Keller and Waller 2002, Reid *et al.* 2005a), so genetic compatibility is frequently interpreted as genetic dissimilarity between members of a mated pair. Heterozygosity has been linked to increased immunocompetence and lower parasite loads in many species such as house finches (*Carpodacus mexicanus;* Hawley *et al.* 2005) and white-crowned sparrows (*Zonotrichia leucophrys oriantha;* MacDougall-Shackleton *et al.* 2005). It is important to note that there is no *a priori* reason to expect male ornaments to advertise genetic dissimilarity to a particular female. Rather, females may assess the genetic dissimilarity, and thus the compatibility of potential mates via alternative cues, for example chemical olfaction (Zelano and Edwards 2002).

Birdsong is an ornament that can convey a large amount of information (reviewed by Searcy and Nowicki 2005). In particular, song complexity has been linked with male condition indicators such as higher body condition and increased immunocompetence (Reid *et al.* 2005a, Reid *et al.* 2005b). Song complexity is often measured by the number of syllables or songs in a male's repertoire and has been heavily implicated in mate choice in many songbird species, including song sparrows (*Melospiza melodia;* Searcy 1984), pied flycatchers (*Ficedula hypoleuca;*

Lampe and Saetre 1995) and great reed warblers (*Acrocephalus arundinaceus;* Hasselquist *et al.* 1996). However, the benefits that females receive by preferentially mating with complex singers are not yet completely understood.

The developmental stress hypothesis (Nowicki *et al.* 1998, Nowicki *et al.* 2002) argues that learned features of song, and thus song learning ability, allow females to infer a male's condition during early life. If so, song learning ability may accurately advertise a male's ability to provide direct (due to carry-over effects of nestling condition into adulthood) and/or indirect (due to good-gene effects on nestling condition) benefits to offspring.

### *Study Species*

Song sparrows are an ideal species in which to test hypotheses related to paternal attractiveness and nestling advantage. Male song sparrows vary considerably in the size of their song repertoires (5-13 in our Ontario study population), and females prefer males with large repertoires (Searcy 1984). In wild-breeding song sparrows, repertoire size has been implicated in male mating success (Reid *et al.* 2004), and is associated with higher immunocompetence (Reid *et al.* 2005a) and superior body condition (Pfaff *et al.* 2007). Individual genetic diversity is associated with greater song complexity (Reid *et al.* 2005a, Pfaff *et al.* 2007), immunocompetence (Reid *et al.* 2005a) and longer territory tenure (Reid *et al.* 2004). Finally, both males and females in this species provide care to nestlings, and nestling growth rates are positively associated with first year overwinter survival and reproductive success (Hochachka and Smith 1991).

### *Objectives and Predictions*

My objective in this section of my thesis was to determine the relationship (if any) between male attractiveness (as measured by song complexity and genetic dissimilarity between social mates) and offspring performance (as measured by nestling growth rates) in song sparrows. If song complexity is an honest advertisement of direct and/or indirect benefits, then males with larger repertoires should have faster growing offspring compared to males with smaller repertoires. Such a relationship could arise from a variety of mechanisms, including good genes effects on offspring viability, a greater ability of complex singers to defend high quality territories, and/or increased parental effort by complex singers and/or their mates. I also predicted that offspring of genetically dissimilar ('compatible') mates should grow at faster rates than offspring of less compatible mates. Such a relationship could arise from compatible-genes effects on offspring viability, and/or increased parental effort by males and/or females paired with genetically dissimilar social mates.

If male attractiveness (as determined by song repertoire size and genetic dissimilarity from their social mate) is positively related to nestling growth rates, three main hypotheses might explain such a pattern. First, a male's song complexity may honestly advertise paternal investment in offspring (Buchanan and Catchpole 2000); this idea will be termed the "good father" hypothesis. Because song sparrows have a high level of biparental care (Smith *et al.* 1982), this may be an important consideration. Second, females may adjust the levels of parental care they provide in response to the attractiveness of their mate, as evaluated by song complexity and/or genetic compatibility; this idea will be termed the "motivated mother" hypothesis. This strategy might be favored if highly ornamented males provide little care to offspring (e.g. Freeman-Gallant 1998), requiring their mates to compensate (Burley 1988), or if the offspring of highly ornamented males are more valuable to females. Third, nestlings that have inherited good and/or compatible genes may grow more quickly regardless of parental feeding rates; this idea will be termed the "good offspring" hypothesis. These three hypotheses are not mutually exclusive.

## Methods

### *Study Site and Population*

I carried out field observations for this study from April – June 2007 and 2008 at the Bracken plot of the Queen's University Biology Station, near Newboro, Ontario, Canada (44° 38' 60 N, 76° 19' 0 W). The song sparrows (*Melospiza melodia melodia*) breeding at this site have been studied by our research group since 2002. Like other populations of song sparrow (e.g. O'Loghlen and Beecher 1999), the study population is socially monogamous but moderate levels of extra-pair paternity do occur (5-20% of nests).

At the start of each field season, adult birds were captured in mist nets or seed-baited treadle traps and provided with a uniquely numbered leg band (Canadian Wildlife Service), as well as a unique combination of coloured leg bands to permit individual identification in the field. A small blood sample (<25μL) was collected from the brachial vein for subsequent genetic analysis.

### *Song recording and analysis*

Song recording and analysis was conducted as described in Chapter 2. All adult males' song repertoires were recorded using Telinga Twin Science Pro parabolic microphone with Marantz Professional PMD 671 digital recorders. An entire repertoire was considered successfully recorded if at least 300 consecutive or 450 non-consecutive songs were captured. Because song sparrows do not learn any new songs after their first year of life (closed-ended learners: Nordby *et al.* 2002), there was no need to re-record returning males every year.

To determine song complexity, I imported all the recordings of each male into SYRINX version 2.6h (John Burt; www.syrinxpc.com) to generate sound spectograms, or visual representations of each song. I first classified all spectrograms into distinct song types through visual inspection and sorting following Pfaff *et al.* (2007). For each song type, I then identified the component syllables, defined as one or more notes that always occur together (Stewart and MacDougall-Shackleton 2008). I defined syllable repertoire size as the number of distinct syllables within a male's repertoire (thus syllables common to multiple song types within a male's repertoire were counted only once). Song repertoire size and syllable repertoire size are highly correlated ($R^2 = 0.365$ $P = > 0.001$, Figure 3.1) and both are widely used as measures of song complexity. For this study I used syllable repertoire size as my measure of song complexity because it is distributed more continuously than song repertoire size.

### *Nestling growth rates*

Members of the research team located nests through behavioral observations and monitored each nest for hatching. Song sparrows attempt multiple nests each year (Arcese *et al.* 2002) so to minimize seasonal effects on nestling growth and parental provisioning I studied only the first breeding attempt for each pair that survived until hatch. On the second day after hatching, I collected a small blood sample (<25uL) from each nestling for genetic analysis. During the same nest visit I also measured each nestling's mass to the nearest 0.1g using a spring scale. I used a felt-tip marker to mark each nestling's toes in a unique pattern so that individuals could be re-identified later in the nestling period. On the sixth day after hatching, I returned to the nest and re-weighed all surviving nestlings to the nearest 0.5g using a spring scale. Nestling growth rate was calculated in grams per day, as: (mass on day 6 – mass on day 2)/4 days. I then calculated the average growth rate of all nestlings in the nest.

### *Cross-fostering experiment*

In 2008 in an attempt to separate genetic from environmental effects on nestling growth rates, I performed a reciprocal cross-fostering experiment. Whenever two nests both hatched on the same day, one nestling from each nest was quickly and gently transferred into the other nest. This swap was performed on day 2 after hatch, immediately after nestlings had been marked, bled and weighed as described above. I then re-visited the nest on the sixth day after hatch, and re-weighed nestlings as described above.

### *Nest visitation rates*

In 2008, I also investigated nest visitation rates of male and female song sparrows. Breeding adults were provided with a plastic leg band to which a small (12mm long x 2mm diameter) Passive Integrated Transponder (PIT) tag (Biomark, OR) was attached. Each tag had a unique number combination that could be read by an antenna and recorded by a receiver. On the third morning after hatching, an antenna and receiver set, wrapped in camouflage tape, was placed unobtrusively within 20cm of the nest in order to record the number and duration of nest of visits by each parent. The receivers were all put at the nest at approximately 5:00am and remained at the nest from 6 to 8 hours (dependant on battery life of the receiver), regardless of weather. I compared abandonment rates across all nests without receivers (2007) and with receivers (2008) and I found no effect of the receiver set on nest abandonment by the parents ($\chi^2 = 0.44$, $P = 0.51$). In determining parental investment, nest visits were measured hourly and taken as total number of visits to the nest per hour. Number of nestlings in the nest was initially included as a possible covariate, however the data did not change significantly whether the hourly visits were taken in terms of visits per hour per nestling or simply visits per hour. I concluded that visitation rate independent of clutch size would better indicate the current level of parental investment; thus, visitation rates presented below are independent of number of nestlings in the nest.

### *Genetic methods*

Once collected, blood samples were allowed to dry onto high wet strength filter paper and stored desiccated for several weeks awaiting laboratory analysis. In the lab, I isolated DNA using an ammonium-acetate based protocol to salt out proteins, modified from Laitinen *et al.* (1994). I genotyped all adults and nestlings using seven hypervariable microsatellite loci: Mme2, Mme7 (Jeffrey *et al.* 2001), Escu1 (Hanotte *et al.* 1994), Pdou5 (Griffith *et al.* 1999), SOSP3, SOSP13, and SOSP14 (Lukas Keller, pers. comm.). PCR was conducted as described in Chapter 2.

### *Parental genetic compatibility*

I used the program MARK (Kermit Ritland, http://genetics.forestry.ubc.ca/ritland/programs.html) to calculate Wang's (2002) coefficient of relatedness between each adult and its social mate, based on microsatellite profiles. Wang's coefficient ranges from -1 to 1, with more negative coefficients representing less relatedness between individuals, and more positive coefficients representing higher levels of relatedness.

### *Nestling genetic diversity*

I estimated the genetic diversity of nestlings using HL (homozygosity by loci; Aparicio *et al.* 2006) as calculated by the macro IRMacroN4 (William Amos; http://www.zoo.cam.ac.uk/zoostaff/amos/#ComputerPrograms) Homozygosity by loci calculates individual genetic diversity by weighting each locus according to its population-wide allelic variability (Aparicio *et al.* 2006).

### *Parentage analysis*

I used microsatellite profiles of all individuals to identify any extra-pair offspring and assign paternity. I categorized nestlings as 'mismatched' if their genotypes were incompatible with those of their social parents at one or more loci. In all cases, the genotypes of mismatched nestlings were compatible with those of the attending females, confirming that these nestlings resulted from extra-pair mating rather than from conspecific brood parasitism.

### *Statistical Analysis*

I used linear multiple regressions to examine the relationships between syllable repertoire size and nestling growth rate, and between parental relatedness and nestling growth rate. I also used multiple regressions to examine the relationship between syllable repertoire size and parental visitation rate on day 3, and between parental relatedness and parental visitation rate on day 3.

High levels of nest predation (45.2% of nests failed before day 6) and low levels of hatching synchrony (48.4% of hatched nests in 2008 could not be included in the cross-fostering experiment) resulted in a low sample size (N = 8 pairs) for which both the cross fostered nestlings survived until day 6 after hatch. Thus, inferential statistics comparing growth rates for cross-fostered nestlings are inappropriate. Instead, I considered the subset of nestlings (n=25) for whom the genetic sire was different from the male in whose nest they were raised. This included extra-pair offspring (n=11) as well as nestlings swapped out of their original nest (n=14). For this subset of nestlings, I used multiple regression to investigate the relationship between growth rate and a) genetic father's syllable repertoire size, and b) parental relatedness between true genetic parents.

All data used for parametric tests were checked for normality using Kolmogorov-Smirnov tests, and found to be normally distributed, and all statistical tests were two tailed.

## Results

I collected nestling growth rate data from a total of 30 nests surviving to six days after hatch (16 nests, including 58 nestling in 2007 and 14 nests, including 59 nestlings, in 2008). Nestling growth rates were higher in 2008 than in 2007 (t = 3.52, df=29, P = 0.001), so I included year as a blocking factor in all subsequent growth rate analyses. The average brood size across both years combined was 3.90 (SE = 0.17).

### *Nesting growth rates (2007)*

Male song complexity (syllable repertoire size) was positively and significantly predictive of the average growth rate of his social offspring between day 2 and day 6 (β = 0.301, df = 21, P = 0.047, Figure 3.2a). Parental relatedness, as measured by Wang's (2002) coefficient of relatedness, was significantly negatively associated with average nestling growth rate ($\beta = -0.498$, df = 21, $P = 0.002$, Figure 3.2b). The entire model (which included syllable number, year and coefficient of relatedness) was significantly predictive of growth rate ($r^2 = 0.647$, df = 3,21, $P < 0.001$).

Table 3.1 – Descriptive statistics of biological data collected from song sparrow population 2007-2008

| | **Mean ± S.D. (n)** | **Range** |
|---|---|---|
| Male syllable repertoire size | 39.2 ± 6.4 (45) | 28 - 56 |
| Relatedness (Wang 2002) between social mates | -0.015± 0.066 (52) | -0.127 - 0.146 |
| Nestling homozygosity by loci | 0.20 ± 0.14 (167) | 0.00 - 0.57 |
| Nestling mass day 2 (g) | 4.4 ± 1.2 (167) | 2.0 - 7.8 |
| Nestling mass day 6 (g) | 14.1 ± 1.5 (117) | 9.5 - 17.5 |
| Nestling growth rate (g/day) | 2.4 ± 0.3 (117) | 1.0 - 3.8 |
| Paternal nest visits/hour on day 3 | 6.2 ± 3.2 (17) | 1.5 - 11.1 |
| Maternal nest visits/hour on day 3 | 18.1 ± 7.0 (15) | 7.5 - 31 |
| Total parental nest visits/hour on day 3 | 23.6 ± 8.5 (13) | 9.5 - 34.6 |

*Parental visitation rates (2008)*

Song complexity (syllable repertoire size) was positively and significantly related to the rate at which males visited their nests on day 3 ($\beta = 0.507$ df = 13, P = 0.049, Figure 3.3a). I did not observe any relationship between male feeding rates and genetic dissimilarity between social mates ($\beta = -0.343$, df = 13, P = 0.164, Figure 3.3b).

Song complexity of the social mate did not significantly predict rates at which females visited their nests on day 3 ($\beta = -0.062$, df = 11, P = 0.817, Figure 3.4a). There was, however, a significant negative correlation between visits to the nest by the mother and parental relatedness ($\beta = -0.642$, df = 11, P = 0.035, Figure 3.4b). Nestling growth rate was not significantly related to maternal visits ($R^2 = 0.290$, P = 0.071) or paternal visits ($R^2 = 0.244$, P = 0.177), however when total visits of both parents were put together, these results approached significance ($R^2 = 0.512$, n = 7, P = 0.071, Figure 3.5).

Extra-pair paternity was found in 18.8% percent (3/16) of nests in 2007 and 21.4% of nests (3/14) in 2008. Overall, 13.8% (8/58) of all nestlings in 2007 were extra-pair, as were 5.1% (3/59) of all nestlings in 2008. There were not enough data to be able to identify the differences in song complexity between genetic and social fathers of these young. I found no difference in growth rate between extra-pair young and within-pair young for both years combined (t = 0.385, df = 112, P = 0.701).

In an attempt to identify any genetic effects on growth rates, I pooled all nestlings with mixed parentage results (both extra-pair young and young that had been swapped from their original nests) and excluded nestlings for which the genetic and social father were the same male. I found no significant relationship between genetic father's song complexity ($R^2 = 0.021$, $P = 0.634$, Figure 3.6a) and nestling growth rate. There was also no relationship between genetic parents relatedness and offspring growth in this group ($R^2 = 0.003$, $P = 0.810$), Figure 3.6b. Parental relatedness is known to be correlated with offspring homozygosity ($R = 0.276$, $df = 159$, $P = 0.0004$), and there was no correlation found between offspring HL (homozygosity by loci) and growth rate in this subgroup ($R^2 = 0.000$ $P = 0.878$, Figure 3.7).

I found no evidence that the presence of extra-pair young in the nest was associated with differences in either maternal effort ($t = 0.705$, $df = 13$, $P = 0.494$) or paternal effort ($t = 0.029$, $df = 16$, $P = 0.977$).

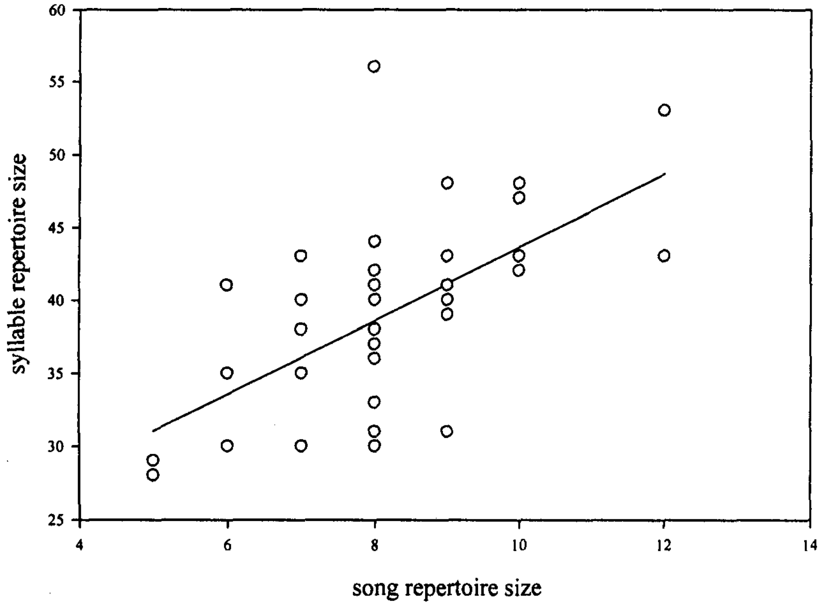

Figure 3.1 – Correlation between male song repertoire size and syllable repertoire size; each plot represents one male. Subsequent analyses use syllable number as the main indicator of song complexity as it provides a more continuously distributed variable.

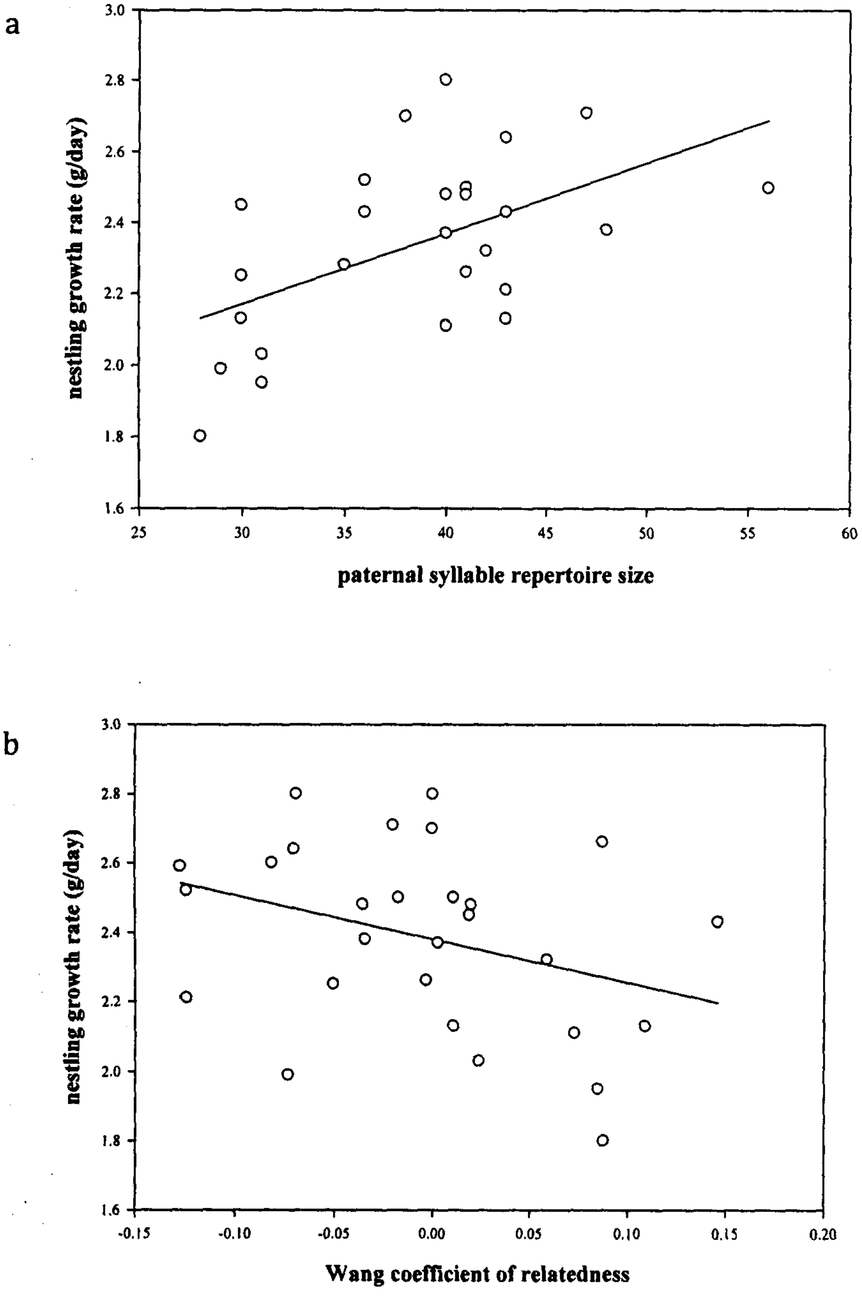

Figure 3.2 – Relationship between nestling growth rates and a) social father's syllable repertoire size and b) social parents' relatedness (Wang 2002) in 2007. Each data point represents the mean nestling growth rates in one nest, and a least-squares regression is included for purposes of illustration.

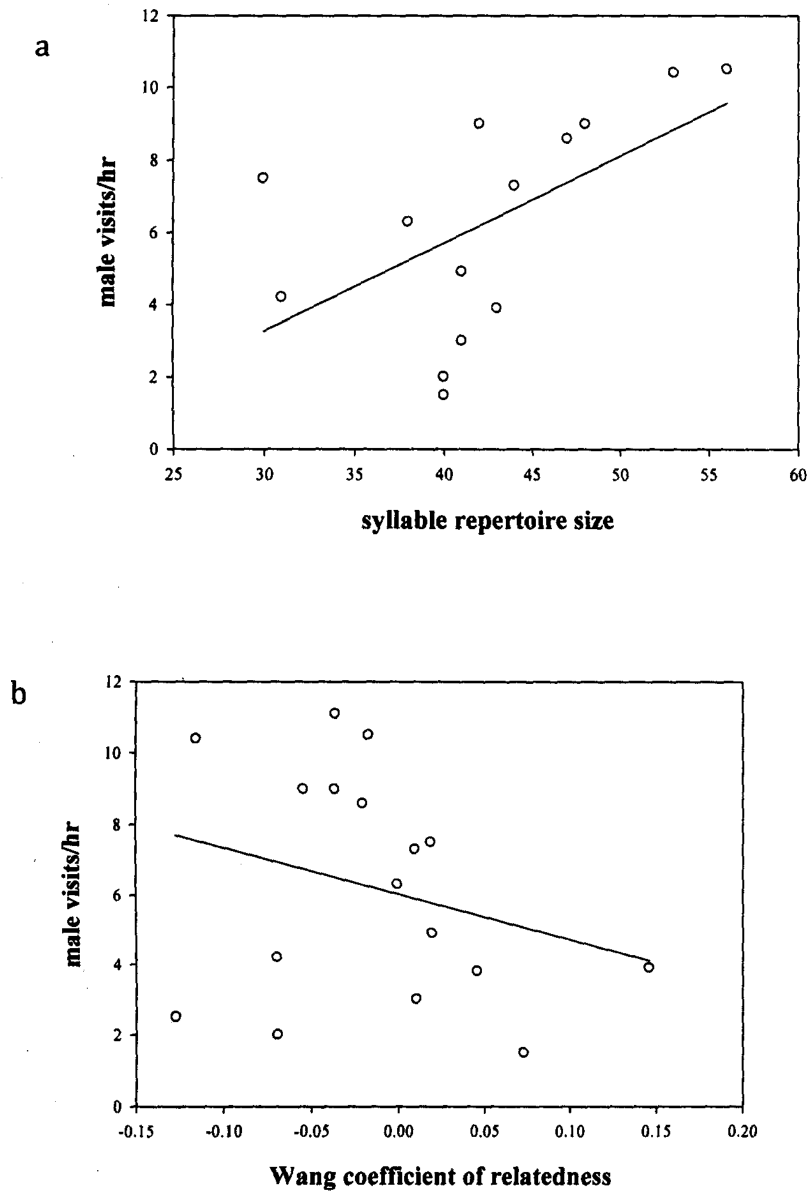

Figure 3.3 – Relationship between a) father's song complexity (syllable repertoire size) and b) parental relatedness on number of hourly paternal visits. The number of visits by the father was significantly correlated with syllable number, however was unrelated to genetic dissimilarity between mates. A least-squares regression is plotted for purposes of illustration.

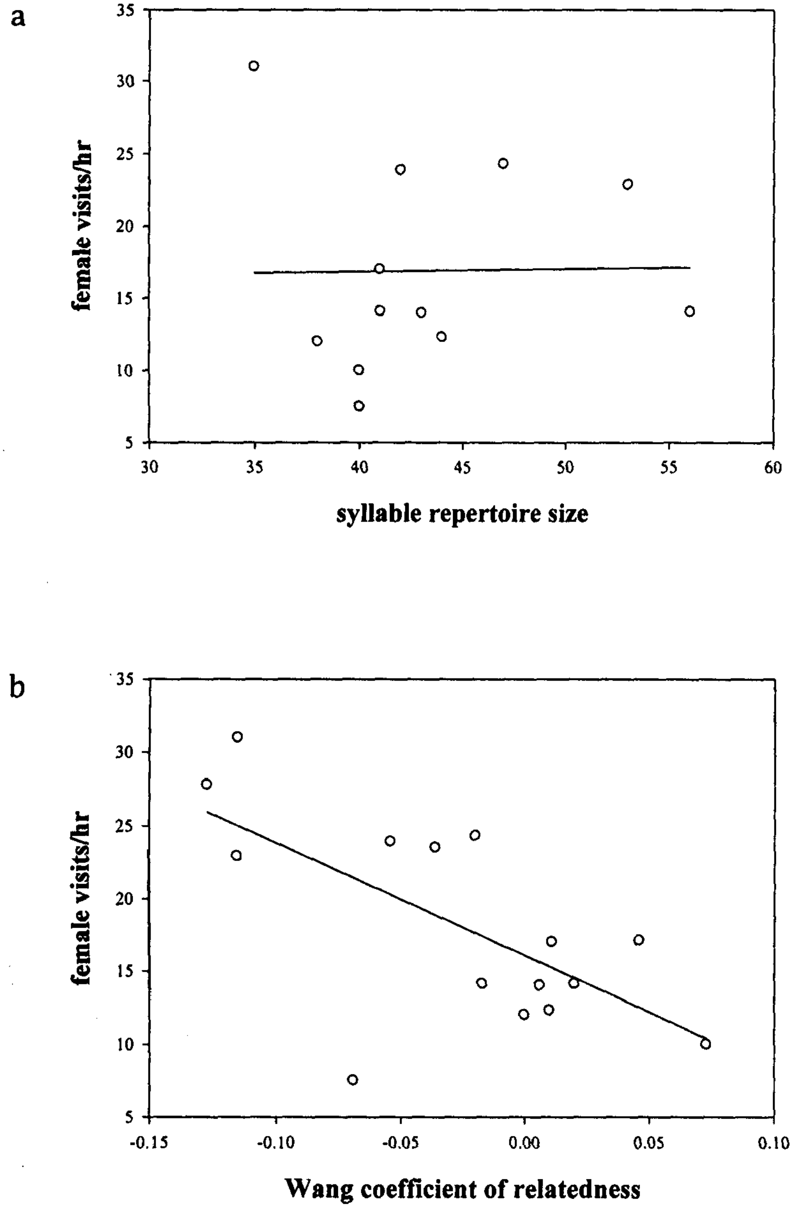

Figure 3.4 – Effect of a) syllable repertoire size of social mate and b) parental relatedness on number hourly maternal visits to the nest. Maternal effort was significantly related to parental relatedness, however number of maternal visits remained unaffected by song complexity of social mate. A least-squares regression is plotted for purposes of illustration.

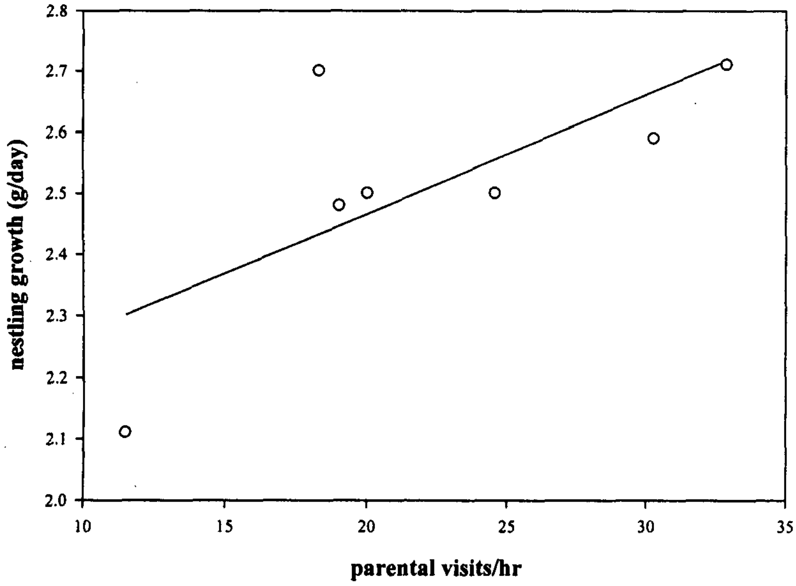

Figure 3.5 – Relationship between total parental visits/hour and average nestling growth rate. Each point represents the mean nestling growth rate in one nest.

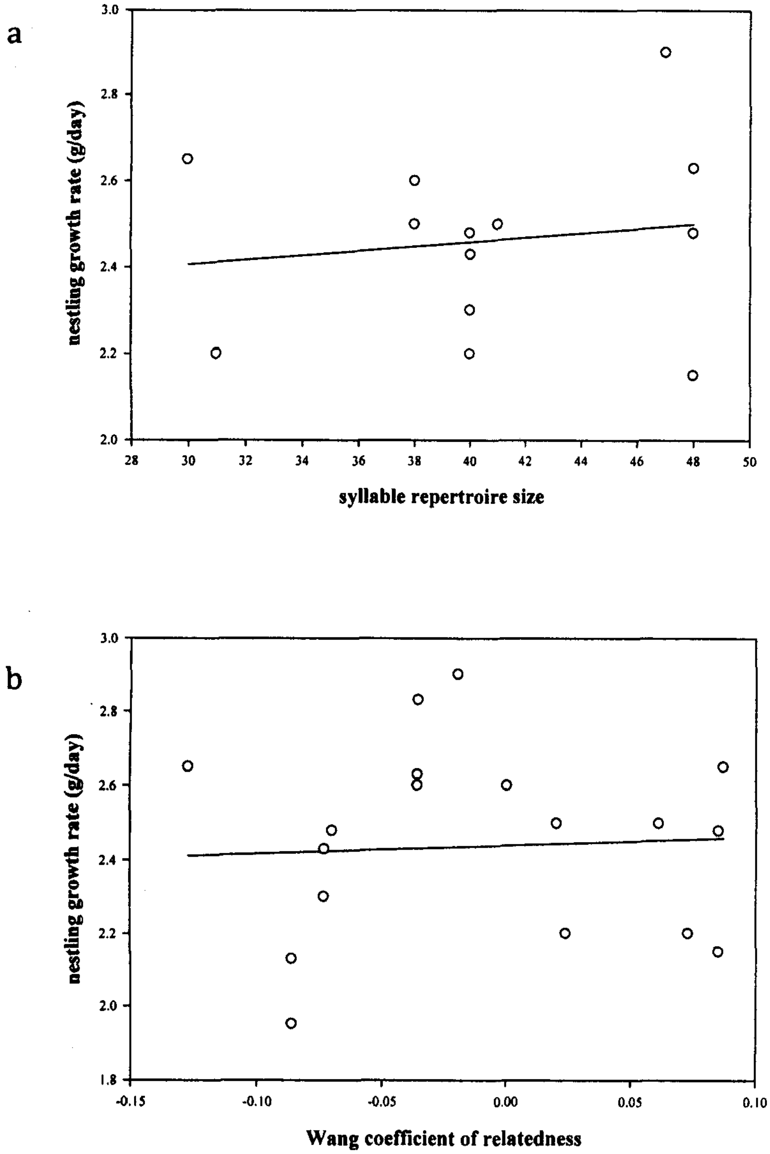

Figure 3.6 – Relationship between nestling growth rate and a) genetic father's song complexity (syllable repertoire size) and b) genetic similarity (Wang 2002) between genetic parents. Each point represents one nestling.

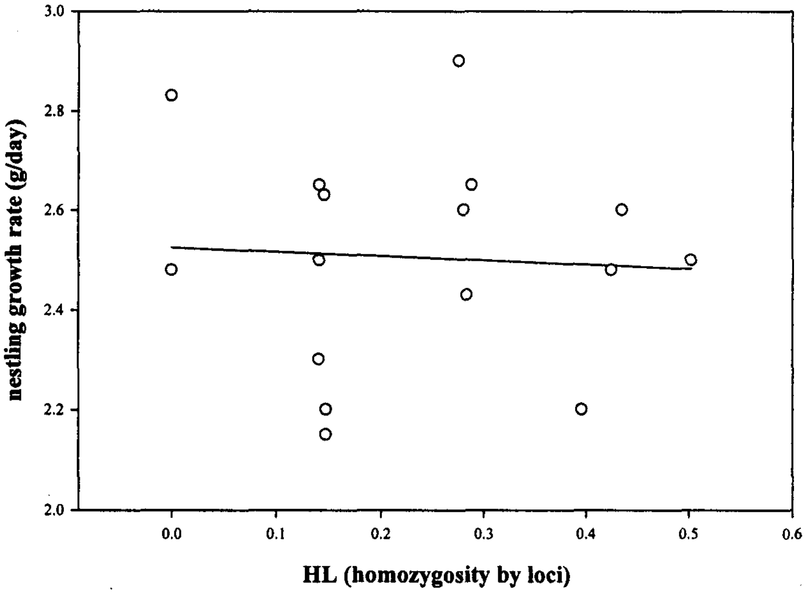

Figure 3.7 – Relationship between nestling homozygosity by loci (HL: Aparicio *et al.* 2006) and growth rate. Only nestlings sired by males other than their social father (products of extra-pair paternity and swapped nestlings) were included in this analysis, each data point represents an individual nestling.

## Discussion

I found compelling evidence that offspring in the nests of more attractive males have an advantage in growth. Nestling growth rate was positively related to social father's song complexity, suggesting that female song sparrows are gaining either direct and/or indirect benefits by mating with more attractive males. Furthermore, female song sparrows may also benefit by mating with genetically dissimilar mates, because nestlings at nests attended by genetically dissimilar adults ('compatible' social parents) grew faster than nestlings at nests attended by genetically similar adults ('less compatible' social parents: Figure 3.2b).

In terms of the mechanism that might be causing this variability in nestling growth rates, I found that male attractiveness (song complexity) was positively correlated with male parental effort (Figure 3.3a), supporting the "good father" hypothesis: song may be an indicator of paternal effort. My findings also provide some support for the "motivated mother" hypothesis due to the negative relationship between female parental effort and the genetic similarity of social mates (Figure 3.4b). Females may be adjusting parental effort in response to the expected heterozygosity of their young. In contrast, my findings provide little support for the "good offspring" hypothesis, as I could detect no effects of genetic father's song complexity, genetic parents' relatedness or offspring homozygosity on nestling growth rate (Figure 3.6, 3.7). Instead, the observed differences in nestling growth rates appear more likely attributable to differences in parental investment.

Nestling growth rate is an important indicator of future success (Hochachka and Smith 1991, Nowicki *et al.* 2002) and therefore and important contributor to parental fitness. However, parental investment is costly and therefore there may be different strategies of investment being used by each parent. My findings that complex singers provide higher levels of care to offspring (Figure 3.3a) are consistent with those of Voltura *et al.* (2002), who found that attractive male house finches invested more in offspring than their less attractive counterparts. Similarly, in sedge warblers (*Acrocephalus schoenobaenus*) song complexity predicts male parental effort (Buchanan and Catchpole 2000). Both of these studies support the idea that males may be advertising quality or ability to care for offspring to females through ornamentation. Gilbert *et al.* (2006) also found a correlation between offspring growth rates and male attractiveness in zebra finches (*Taeniopygia guttata*), apparently because offspring of attractive males were more effective beggars.

Many other studies have found the opposite effect of male attractiveness on parental effort. If an attractive male trait is very costly to display, it may be associated primarily with more indirect rather than direct benefits. Decreased paternal care by attractive males is also found in species where there is a high incidence of extra-pair paternity (Møller 2000), and more attractive males increase their reproductive success by spending time and energy on pursuing extra-pair copulations. It often follows, then, that females respond to this attractiveness and lack in parental care by increasing their own investment in offspring. Support for the "differential allocation hypothesis" (Burley 1988) comes from studies of barn swallows (*Hirundo rustica;* de Lope and Møller 1993), savannah sparrows

(*Passerculus sandwichensis;* Freeman-Gallant 1998) and bluethroats (*Luscinia svecica;* Rohde *et al.* 1999), among other species. In our population of song sparrows, song complexity is considered an honest indicator because it is indicative of developmental stress and condition rather than current cost (Nowicki et al 2002), and extra-pair paternity in our population is relatively low, which is probably why we do not see differential allocation by females but rather an increase in paternal effort with increased male attractiveness.

Many studies have found a similar effect of perceived male attractiveness on female parental effort, however when examined closely, it is usually a result of differential allocation (Limbourg *et al.* 2004, Gorman *et al.* 2005). Studies that have found a female investment response to male attractiveness regardless of male parental effort include blue-footed boobies *Sula nebouxii* (Velando *et al.* 2006), and peafowl *Pavo cristatus* (Petrie and Williams 1993). Although there have been studies in other taxa that have found an increase in female parental effort in response to perceived genetic quality of their offspring (Kotiaho *et al.* 2003), this is the first study to my knowledge to find a relationship between female parental effort and genetic similarity between mates in an avian species. However, if there is a heterozygote advantage for offspring, it is not surprising that females would increase parental effort in response to the individually "attractive" trait of genetic compatibility.

I found little evidence for either good genes or the effect of compatible genes on offspring growth rates (Figure 3.6, Figure 3.7). Instead, the superior performance of nestlings on the territories of complex singers, and of nestlings attended by genetically dissimilar social parents, appears to be due largely to differences in paternal feeding rates. Similar results have been found by Kunz and Ekman (2000) in blue tits *Parus caeruleus.* In this species, although there are measureable heritable traits nestlings receive from parents, nestling growth itself depends heavily on parental feeding rates. A cross-fostering experiment on European starlings *(Sturnus vulgaris)* also reached similar conclusions (Rickleffs and Peters 1981). However, offspring of complex singers and/or of genetically dissimilar parents may receive indirect benefits that are not associated with growth rate during the nestling period. Instead, other elements of fitness (such as increased immunocompetence, ability to respond to stress and structural body condition) may still be gained though parental genetic quality. These effects may also become more apparent as nestlings reach adulthood, or when food is less abundant (Hegyi *et al.* 2006).

This study shows the direct parental benefits female song sparrows receive by mating with males with higher song complexity. This fits well with the current literature (Møller 2000, Buchanan and Catchpole 2000, Nowicki *et al.* 2004), and confirms results found in a closely related species, savannah sparrows *Parsserculus sandwichensis* (Freeman-Gallant 1996), where females prefer males that provide more parental care.

The limitations of this study are consistent with many studies that are based on observations and natural correlations rather than experiments. Although there are many advantages to observing wild behavior in natural populations, laboratory experiments are often better able to isolate causal relationships. Many variables such as territory quality and food quality could not be examined. Territory quality especially may be correlated with mate attractiveness, and therefore may also mediate the relationship seen between male attractiveness and feeding rates. As I discovered, it is quite difficult to obtain a large enough sample size for experiments when it is dependent on the natural survival (and hence predation avoidance) of nestlings. It would be useful to attempt more cross-fostering experiments in future years to increase this sample size, and a common-garden experiment to examine the heritability of repertoire size would also be valuable.

This study contributes to the extensive literature examining female preferences and benefits in song sparrows. I have shown that male song complexity as well as parental relatedness are correlated with offspring growth rates, that male song complexity is a good predictor of male parental effort through feeding, and that females may be altering their own investment in response to mate compatibility. Consistent with many studies, I did not find any genetic effects of parental attractiveness or compatibility on offspring growth per se. As we become more familiar with the direct benefits associated with female choice and mate attractiveness in song sparrows, we will be able to form a complete picture of the forms of sexual selection acting in this species.

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## *Chapter 4:* Conclusion

My thesis research examined several behavioral strategies that might be used by female song sparrows to increase reproductive success. First, I found that females appear to adjust their mating tactics (production of extra-pair offspring) in response to the attractiveness of their social mate (Chapter Two). This finding is consistent with studies on many other socially monogamous avian species in which females appear to use extra-pair matings to obtain good genes for their offspring (great reed warblers *Acrocephalus arundinaceus* Hasselquist *et al.* 1996, yellow warblers *Dendroica petechia* Yezerinac and Weatherhead 1997, superb fairy-wrens *Malurus cyaneus* Dunn and Cockburn 1999). Secondly, female song sparrows also appear to enhance the heterozygosity of their young by pursuing extra-pair copulations (Chapter Two). Again, similar patterns have been observed in other songbird species where heterozygosity is advantageous (blue tits *Parus caeruleus* Foerster *et al.* 2003, savannah sparrows *Passerculus sandwichensis* Freeman-Gallant *et al.* 2006). Finally, females paired with more genetically dissimilar social mates visit their nest at higher rates than those mated with males that are more genetically similar (Chapter Three). Thus females may adjust their investment in offspring in response to the perceived heterozygosity of offspring. Similar examples of this behaviour in the literature are restricted to investments other than feeding (such as egg quality, Petrie and Williams 1993) or taxa other than birds (reviewed by Neff and Pitcher 2005), however studies such as these consistently show the advantages of this strategy.

My thesis research also examined the relationship between male attractiveness and paternal behaviour and offspring fitness. Complex song in song sparrows appears to be an honest advertisement of male feeding rates to offspring (Chapter Three). This relationship has also been seen in sedge warblers (*Acrocephalus schoenobaenus* Buchanan and Catchpole 2000), indicating that complex song may advertise direct benefits in the form of paternal care. This ability of attractive males to better provision offspring may also help to explain why complex singers produced more male-biased sex ratios than did their counterparts with smaller song repertoires (Chapter Two): attractive males may be raising sons that are growing faster and hence may be outcompeting daughters. Sons are considered the more expensive sex to produce in many species, due to high energetic costs for size and other developmental factors (e.g. Stamps *et al.* 1987, Weimerskirch *et al.* 2000, Daunt *et al.* 2008).

In contrast, I found no evidence that females manipulate the primary brood sex ratio in response to mate attractiveness (through song complexity or genetic compatibility). Whether this is a result of mechanistic constraint or of lack of selective pressure is unknown, however these findings are consistent with the results of many other avian studies of sex allocation theory (Westerdahl *et al.* 1997, Saino *et al.* 1999 and Leitner *et al.* 2006, but see Ellegren *et al.* 1996 and Dreiss *et al.* 2006).

The behaviours I examined are likely to have important implications for the quality of offspring. Increased parental care by attractive males, as well as by females mated to genetically dissimilar (compatible) social mates, appears to have a positive effect on offspring growth. This may in turn have important carryover effects on adult fitness, including overwinter survivorship (Hochachka and Smith 1991) as well as song learning ability (Nowicki *et al.* 2002).

This study fits well into the literature discussing the developmental stress hypothesis (Nowicki *et al.* 1998). The developmental stress hypothesis states that nutritional (or other) stress in the early development of nestlings may have a profound impact on the development of the song learning structures in the brain, such as the higher vocal centre or HVC (Nowicki *et al.* 2002). This has a direct effect on the learning ability of the individual, such that nestlings that undergo better nutrition and care in the nest are better able to learn complex songs. Since song complexity has been shown to be an important factor of female choice in this species (Searcy 1984), this may directly affect the individual's reproductive success as an adult. There are several points to consider from the perspective of the developmental stress hypothesis in relation to my study.

First, heterozygosity has been found to correlate with song complexity in song sparrows (Reid *et al.* 2005a, Pfaff *et al.* 2007). Song complexity is also correlated with HVC size in the brain (Pfaff *et al.* 2007). Therefore, in order to increase their reproductive success, females may attempt to increase the heterozygosity of their offspring by pursuing extra-pair copulations, especially if mated to a less attractive male. Second, it also stands to reason that females should adjust parental investment in response to the perceived heterozygosity of their offspring. It seems unclear whether heterozygosity is directly affecting HVC size, or whether females are *responding* to perceived heterozygosity, increasing HVC size through higher feeding rates. Controlled laboratory experiments may be required to distinguish between these causal relationships.

Furthermore, better singers being better fathers also fits well with the developmental stress hypothesis (Nowicki *et al.* 1998). The developmental stress hypothesis argues that song complexity is an indicator of nestling environment and development as a whole. Since offspring growth in song sparrows has also been correlated with increased overwinter survival (Hochachka and Smith 1991), and song complexity is also correlated with other measures of current body condition (Reid *et al.* 2005b, Pfaff *et al.* 2007), then it is no surprise that more complex singers are better able to take care of their offspring. In order to further confirm this theory, it would be interesting to examine the impact of male heterozygosity on parental ability.

My findings suggest that male and female song sparrows may have very different parental strategies and abilities. Male parental investment seems to be quite dependent on overall quality, as advertised by song complexity (Chapter Three). Female parental investment, however, seems to be more responsive to the perceived quality of both mates and offspring (Chapter Three). Both appear to have a direct effect on the growth (and in some cases, sex-biased survival) of offspring and in this way may also be increasing the future reproductive success of these offspring.

It is clear that parental investment strategies are an important piece of the evolutionary puzzle. Studies into such strategies provide insight into the behaviours and benefits that are driving sexual selection in many species. As we become more adept at quantifying these behaviours in the field and better able to distinguish between environmental and genetic effects on individual survival and reproduction we may be able to better understand the underlying factors driving evolution.

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