

IDENTIFYING THE ROLE OF SELECTION IN THE EXPRESSION OF  
AGGRESSIVE PHENOTYPES IN SONG SPARROWS, *MELOSPIZA MELODIA*

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## TABLE OF CONTENTS

	Page
List of Tables .....	iv
List of Figures .....	v
List of Abbreviations.....	vi
Abstract .....	vii
Introduction.....	9
Methods.....	12
Study Population and Field Methods .....	12
Aggression Assays .....	13
Microsatellite Genotyping.....	14
Paternity Assignment .....	15
Statistical Analyses .....	18
Results.....	21
Paternity Analysis.....	21
Aggression and Reproductive Variables.....	22
Characterizing Extrapair Paternity.....	23
Aggression and Extrapair Paternity .....	24
Discussion.....	25
Literature Cited.....	29

## LIST OF TABLES

Table	Page
1. Microsatellite loci used for genotyping analyses.....	16
2. Microsatellite characteristics based on 308-325 individuals per locus .....	17
3. Regression analyses relating aggression to annual reproductive success, nest productivity, and nestling growth .....	22

## LIST OF FIGURES

Figure	Page
1. Growth rate has a positive linear relationship with aggression .....	23
2a. A comparison of males that gained at least one extrapair fertilization to males that did not gain extrapair fertilizations.....	24
2b. A comparison of males that lost at least one within-pair fertilization to males that did not lose within-pair fertilization.....	24
3. Pairwise comparisons of within-pair (social) sires to extrapair sires .....	24

## LIST OF ABBREVIATIONS

Abbreviation	Page
1. Extrapair fertilization (EPF) .....	10
2. Within-pair fertilization (WPF) .....	18

## ABSTRACT

### IDENTIFYING THE ROLE OF SELECTION IN THE EXPRESSION OF AGGRESSIVE PHENOTYPES IN SONG SPARROWS, *MELOSPIZA MELODIA*

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Evolutionary theory predicts that selection should minimize variation in behavioral traits if they are associated with reproductive success. Yet, some behavioral traits, such as territorial aggression, show high levels of variation. Selection may play a role in maintaining this variation, if individuals with differing aggressive phenotypes use alternative tactics to obtain similar reproductive success. In this study, we examined the role of selection in the maintenance of individual variation by measuring the fitness consequences of territorial aggression in an urban population of song sparrows, *Melospiza melodia*. Song sparrows are socially, but not genetically monogamous due to the presence of extrapair fertilizations (EPFs). We tested the hypothesis that aggressive male song sparrows would not achieve greater annual reproductive success than unaggressive males due to trade-offs associated with aggressive phenotypes. We predicted: 1) that aggression would show a positive relationship with nest productivity, but that aggressive males would face a trade-off between the energy required to defend high quality territories and paternal investment in nestling growth; and 2) that aggressive males would be more likely to seek or obtain extrapair fertilizations, but that they would face a trade-off between seeking extrapair fertilizations and losing paternity in their social

nests. We did not uncover a relationship between aggression and annual reproductive success or nest productivity, but we did find a significant, positive relationship between aggression and nestling growth rate. Nestling growth rates have been shown to affect both the recruitment and reproductive success of offspring, and thus, aggressive male song sparrows could achieve greater fitness by producing more successful offspring than unaggressive males. Our study indicates that aggressive male phenotypes may be favored via selection on their offspring that acts well after fledging and emphasizes the importance of examining multiple components of reproductive success when investigating the role of selection in maintaining individual variation in behavioral traits.

## INTRODUCTION

Evolutionary theory predicts that selection should minimize variation in behavioral traits if they are associated with reproductive success (e.g. Darwin 1859). Yet, some behaviors show high levels of variation (Dingemanse et al. 2004, Sih et al. 2004, Smith & Blumstein 2008, Schuett et al. 2010). Territorial aggression is a behavioral trait that significantly affects male reproductive success (West-Eberhard 1983, Arcese & Smith 1985, Smith & Blumstein 2008, Schuett et al. 2010, Scales et al. 2013). If aggressive males have greater reproductive success, we would expect behavioral variation in a population to decrease over time (Coleman & Wilson 1998); yet, territorial aggression shows a great deal of individual variation (Nowicki et al. 2002, Tuttle 2003, Dingemanse et al. 2004, Hyman et al. 2004, Sih et al. 2004, Schuett et al. 2010). Selection may play a role in maintaining this variation, if individuals with differing aggressive phenotypes use alternative tactics to obtain similar reproductive success (Tuttle 2003, Smith & Blumstein 2008). In this study, we examined the role of selection in the maintenance of individual variation by relating territorial aggression to reproductive success in socially monogamous song sparrows, *Melospiza melodia*.

Aggression is often favored in territorial birds, because territory settlement is a prerequisite to obtaining a social mate (e.g. Brown 1969, Arcese and Smith 1985). If selection plays a role in the maintenance of individual variation, we would expect directional selection on aggression to be constrained by trade-offs in fitness consequences across contexts (Smith & Blumstein 2008). Territorial aggression in birds has been related to nest productivity via increased pairing success (Duckworth 2006a, Kunc et al.

2006), access to high quality territories (Duckworth 2006b, Scales et al. 2013), and parental investment (Mutzel et al. 2013). However, males may face a compromise between allocating energy to territorial defense and allocating energy to parental care. Multiple studies have supported a trade-off between aggression and parental investment, such as decreased nestling provisioning by aggressive male house sparrows, *Passer domesticus* (e.g. Hegner & Wingfield 1987) and decreased female provisioning during incubation by aggressive male Western bluebirds, *Sialia mexicana* (Duckworth 2006a). A male's investment in parental care has the potential to indirectly affect his fitness, because chicks that grow at a faster rate, or are larger at fledging, have shown greater survival rates and reproductive success (Arcese and Smith 1985, Both et al. 1999, Duckworth 2006a, Class & Moore 2010, Hegyi et al. 2011).

Aggressive behavior may have especially important fitness consequences in bird species that exhibit social monogamy. Most territorial songbirds are socially, but not genetically monogamous due to the presence of extrapair fertilizations (EPFs). Extrapair paternity represents an alternative reproductive strategy in which males seek to increase their fitness by seeking copulations outside of their social bond. However, in some species, the fitness benefits of EPFs are not realized, because males appear to face a trade-off between seeking EPFs and losing paternity in his social nest (Tuttle 2003; Hill et al. 2011). Individual levels of aggression have been shown to predict the likelihood a male will seek or obtain success in EPFs, and the likelihood a male will be cuckolded at his social nest (Mennill et al. 2002, Tuttle et al. 2003, Hill et al. 2011).

Extrapair fertilizations typically make up 20 to 30% of offspring in song sparrows (Hill et al. 2011), and individual variation in aggression has been well described

(Nowicki et al. 2002, Hyman et al. 2004; Hyman & Hughes 2006). Aggressive male song sparrows appear to obtain higher quality territories, and may gain greater reproductive success than unaggressive males, because females on high quality territories lay more eggs (Scales et al. 2013). Aggressive males are also considered more of a threat by territorial males than non-aggressive males, indicating that aggressive males may be more likely to seek territorial intrusions and EPFs (Hyman & Hughes 2006). However, whether aggression correlates with EPFs or reproductive success is not yet known. In this study, we tested the hypothesis that aggressive male song sparrows do not obtain greater reproductive success than unaggressive males due to trade-offs related to the energetic costs of aggression, and the relationship between aggression and patterns of paternity. We predict: 1) that aggression will show a positive relationship with nest productivity, but that aggressive males will face a trade-off between the energy required to defend high quality territories and paternal investment in nestling growth (and thus, recruitment); and 2) that aggressive males will be more likely to seek or obtain extrapair fertilizations, but they will face a trade-off between seeking EPFs and losing paternity in their social nests. By examining how the fitness consequences of territorial aggression, we can better understand the role of selection in maintaining individual variation in behavioral traits.

## METHODS

### *Study Population and Field Methods*

Data were collected from an urban population of song sparrows, *Melospiza melodia*, on the campus of Western Carolina University, Cullowhee, NC (35°18'N, 38°04'W, elevation 640 m) during the breeding season (late March to early August) in 2013. The study site (0.28km<sup>2</sup>) is bounded by four major roads that circle the main campus where the densest population of song sparrows is found. The study site is composed of buildings surrounded by small roads and walkways in which patches of lawn, ornamental shrubs, and scattered trees are found. Despite clear anthropogenic disturbance, this habitat supports a dense population of song sparrows, and provides ample nesting sites, as well as man-made and natural song posts.

Song sparrows are territorial and socially monogamous. Males sing from distinct song posts in order to defend a territory and attract a female (Arcese et al. 2002). A total of 52 territories (186 territories/km<sup>2</sup>) were identified by plotting song posts and by observing agonistic interactions among males. Adult song sparrows were caught using mist-nets and Potter traps. Once caught, they were banded with one U.S. Fish and Wildlife Service band and three colored bands to allow for visual identification at a distance. Blood was drawn from the brachial vein at the time of banding. Adults were considered paired as long as they were pair bonded at some point throughout the breeding season. Unpaired males held a territory, but did not acquire a social mate during the breeding season. Males were considered floaters if they did not holding a territory at any point throughout the breeding season (Sardell et al. 2010). Every paired and unpaired

adult on the study site was sampled: 53 paired females, 54 paired males, and 1 unpaired male. All observed floater males (n=7) were sampled, as the exclusion of floaters biases paternity analysis (Sardell et al. 2010). Territorial take-overs (<4% of territories) and mate switching (<6% of territories) occurred occasionally.

Female song sparrows nest in open cup nests in shrubs and bushes, and can have one to multiple broods throughout the breeding season. Only the female incubates the eggs, while both the male and female share in nestling provisioning (Arcese et al. 2002). Nests were located by observing parental behavior, and territories were visited at least once weekly to monitor nesting status. We defined a successful nest as a nest in which at least one chick “fledged”. We referred to a chick as fledged, if it was alive at the nest on our last visit prior to fledging (6 days old). Every successful nest and the majority of failed nests were located. Nest fate was followed until day 6. We did not disturb the nest after day 6 to minimize the risk of force fledging. A total of 112 nesting attempts were observed, 68% of which were successful (n=76). In order to assign paternity to young, we collected blood from the nestlings at the tarsal vein (n=218) when a nest was located. At 6 days old, nestlings were banded with one U.S. Fish and Wildlife band. If not all nestlings survived to 6 days old, blood was collected again from the surviving nestlings (n=23). A subset of nests (n=32) was weighed every other day until day 6.

#### *Aggression Assays*

We measured aggression using standard playback experiments, in which we simulated a territorial intrusion by broadcasting conspecific song from the center of the focal male’s territory (Nowicki et al. 2002, Hyman et al. 2004, Hyman & Hughes 2006, Scales et al. 2011, Scales et al. 2013). Prior research has shown that the closer a male

approaches the speaker, the more likely he is to attack a taxidermic mount (Searcy et al. 2006), so we used average approach to the speaker (m) as our metric for aggressive behavior. Thus, an aggressive male was defined as a male with a low average approach (Hyman and Hughes 2006). We conducted aggression assays during the hours of peak territorial defense (06:00-11:00 hours). For playback, we used song recorded from northwestern Pennsylvania to eliminate possibility that the test subject knew the singer (for further recording information, see Scales et al. 2013). Distance from the speaker was binned into 0-2m, >2-4m, >4-8m, >8-16m, and >16m, which were marked with flags to assist the observer. Song was broadcast for a total of 6 minutes, and we assigned a distance to the focal male once every 5 seconds for a total of 9 minutes: 6 during playback and 3 afterwards.

#### *Microsatellite Genotyping*

Blood samples were dried and stored at room temperature (20°C) for less than 6 months. DNA was extracted using the Wizard SV Genomic DNA Purification System (Promega Corp., Fitchburg, Wisconsin) and quantified using the NanoDrop 1000 Spectrophotometer v3.8 (Thermo Fisher Scientific Inc., Waltham, Massachusetts). Individuals were genotyped using seven fluorescently labeled microsatellite loci (Table 1): Sosp01, Sosp02, Sosp05, Sosp07, Sosp08 (Sardell et al. 2010), Mme2 and Mme8 (Jeffery et al. 2001). Loci were amplified using polymerase chain reactions (PCR) in three multiples reactions using the methods described by Sardell et al. (2010). Each reaction was carried out at a volume of 10  $\mu$ L containing 1X TE buffer (Tris-EDTA buffer), PCR master mix [1X QIAGEN PCR Buffer, 3mM MgCl<sub>2</sub>, dNTP mix, HotStarTaq DNA Polymerase], 0.2  $\mu$ M primers (each), and 1  $\mu$ L of purified DNA. PCR

profiles consisted of a denaturation step of 95 °C for 15 min to activate the HotStarTaq, followed by 30 cycles of 94 °C for 30 s, the primer specific annealing temperature for 90 s (Table 1), 72 °C for 90 s, and ending with an extension of 72 °C for 10 min. PCR products were then diluted with 24 µL deionized water, and 1 µL of this mixture was added to the size standard (1 µL Georgia Genomics Facility ROX 500 size standard and 9 µL Applied Biosystems Hi-Di Formamide) and subject to 120 s at 95 °C. Fragment lengths were analyzed using capillary electrophoresis (Applied Biosystems 3130 Genetic Analyzer and Applied Biosystems 3730xl DNA Analyzer). Genotypes were assigned using GeneMapper v3.7. All electropherograms were checked by eye.

#### *Paternity Assignment*

The seven loci used in this study were characterized using CERVUS 3.0.6 (Table 2). The proportion of loci typed was 0.9491. CERVUS uses a goodness-of-fit Hardy-Weinberg equilibrium test with a Bonferroni adjustment to compare expected and observed heterozygosity (Marshall et al. 1998, Lessios 1992). Most loci did not differ significantly from Hardy-Weinberg equilibrium and had low null allele frequencies ( $F_{\text{null}} < 0.03$ ). Only one locus (Mme2) differed significantly from Hardy-Weinberg equilibrium, indicating the presence of null alleles ( $F_{\text{null}} = 0.0695$ ). The frequency of null alleles at this locus was taken into account during paternity assignment (Pemberton et al. 1995). Mean genotyping error rate across loci was less than 2%, so overall genotyping error due to allelic dropout or false alleles was low. The combined paternal exclusion probability given a known mother was 0.995. CERVUS uses maximum likelihood analysis to assign parentage. Males are assigned a logarithm of odds (LOD) score

Table 1. Microsatellite loci used for genotyping analyses.

Locus	Microsatellite	Primer sequence	T <sub>a</sub> °C	GenBank accession number
Sosp01	(GGAT) <sub>17</sub> GCAT (GGAT) <sub>2</sub>	F: GCCAACACCCTCAACAAGAT R: ACCAACTGATGCACCTTCTG	64	GU301255
Sosp02	(CTGT) <sub>6</sub> (GT) <sub>3</sub>	F: AA ACTCGCGTCTTTGCTAGG R: CAGGTGTCCTGCAGATGTTG	64	GU301256
Sosp05	(GACA) <sub>2</sub> GA CT (GACA) <sub>8</sub>	F: GAAGGTGTTGGTGCTCACAG R: CTCCTGGGCCAGACAAAG	58	GU301259
Sosp07	(GACA) <sub>8</sub>	F: GTTCCGAGCCCATCCATCT R: CTCTGAGCCCTGCGTTGT	58	GU301261
Sosp08	(GTCT) <sub>5</sub>	F: GTCCTTGGAGTTTGCAGGTATC R: CCTGCAAAAGTAAGAAAGAGAGG	58	GU301262
Mme2	(TG) <sub>30</sub>	F: ATCAGAGATTCTGCTACACACCC R: GAAATTGTATCCGCCACCTCATTC	63	AF127377
Mme8	(TG) <sub>3</sub> TC (TG) <sub>13</sub>	F: TCATGGAGATGGGTGAATGCC R: TGAATCAGCAGCACACAACC	63	AF127382

based on mother-father-offspring mismatches and taking into account genotyping error.

Since in some cases, multiple males had positive LODs, the parameter, Delta, defined

Table 2. Microsatellite characteristics based on 308-325 individuals per locus.

Locus	Number of alleles	H <sub>o</sub>	H <sub>e</sub>	Probability of paternal exclusion	Null allele frequency	Genotyping error rate
Sosp01	31	0.858	0.903	0.804	0.024	0.000
Sosp02	5	0.631	0.635	0.397	0.005	0.000
Sosp05	10	0.642	0.668	0.466	0.022	0.010
Sosp07	6	0.541	0.545	0.312	0.011	0.000
Sosp08	3	0.063*	0.061	0.030	-0.008	0.000
Mme2	12	0.714**	0.820	0.645	0.070	0.084
Mme8	12	0.769	0.812	0.638	0.027	0.011
Combined				0.995		

\*Statistical deviation from H-W equilibrium was not tested at this locus due to too few alleles.

\*\*Significant deviation from H-W equilibrium at this locus.

as the difference between the LOD of the most likely and second most likely sires, was used to assign paternity. If only one male had a positive LOD, the Delta score was equal to the LOD score. If no males had a positive LOD, then Delta was undefined. A simulation of paternity analysis was used to assign confidence to the most likely sire based on Delta scores. Genotypes were simulated for 10,000 offspring using an estimated 61 candidate fathers (95% of the candidate fathers sampled). The population was not insular, and so some unidentified floater males and males on territories bordering the study site were not sampled. The proportion of loci typed was 0.949, and an error

rate of 0.015 was estimated from mother-offspring mismatches. A strict confidence level of 95% and a relaxed confidence level of 80% were set for population-level probabilities (Marshall et al. 1998, Kalinowski et al. 2007).

Based on the results of the maximum likelihood analysis, the majority of extrapair sires (96%) were located within two territories of the chick in question. These results parallel those seen in prior studies on extrapair paternity in song sparrows (Sardell et al. 2010, Hill et al. 2011). This information was used to inform the CERVUS assignments in a “total evidence” approach (see Prodohl et al. 1998). In most cases, the CERVUS assignment (the male with the highest likelihood score) was accepted as long as the male mismatched the chick at  $<2$  loci; however, we rejected the CERVUS assignments when 1) the most likely sire was located  $>2$  territories away of the focal chick, or 2) when the two males had similar likelihood scores and were located within 2 territories of the focal chick. In these cases, the second most likely sire was assigned if he: 1) was the social father or was located  $\leq 2$  territories of the focal chick, and 2) had fewer mismatches, or a mismatch congruent with a null allele (sire and offspring are homozygous for different alleles). We also used this approach to assign paternity by eye to the few offspring to which CERVUS did not assign paternity with statistical confidence. If these criteria were not met, paternity was considered undefined (Webster et al. 2004, Sardell et al. 2010, Hill et al. 2011).

### *Statistical Analyses*

Within-pair fertilizations (WPFs) were defined as the number of offspring sired by the social male. Extrapair fertilizations (EPFs) were defined as the number of offspring sired by a male other than the social male (Sardell et al. 2010). Because our

data were collected from an open population, it is possible we missed EPFs by males that held territories close to the edge of the study site. We estimated annual reproductive success per male by his total number of chicks sired that were still alive the last day we visited nests prior to fledging (day 6).

We measured nest productivity as the number of 6-day-old chicks per successful nest. We used chicks alive in the nest at day 6, the last day we visited the nest prior to fledging, as an estimate of the number of fledglings, and we will use the word fledgling to refer to 6-day-old chicks for the duration of the study. As a further gauge of within-pair reproductive success, we estimated recruitment probability by measuring the trajectory of nestling growth in a subset of nests ( $n=32$ ). First, we identified the overall shape of the growth curve by plotting a best-fit line to average daily masses (days 0-6) of all nestlings sampled. An exponential curve best fit our data. The exponential model was justified given that we measured the early stage of nestling growth, and song sparrows, when measured until day 11, show the sigmoidal pattern of growth commonly seen in altricial nestlings (Ricklefs 1984, Sogge et al. 1991).

We used linear regression analyses to relate aggression (average approach) to annual reproductive success, nest productivity, and nestling growth rate. We evaluated residuals of regression for linearity and normality, and log transformed data when appropriate. In order to illustrate the effect of aggression on nestling growth rate, while controlling for the affect of sampling date (Julian hatch date) we analyzed approach score and hatch date as two potential explanatory variables in a multiple linear regression. To characterize extrapair paternity in our population, we used: 1) a Pearson's chi-squared test to examine whether the likelihood of a male to seek out EPFs related to his likelihood

of cuckoldry, and 2) a t-test to evaluate if males that gained EPFs had greater mean annual reproductive success. To relate EPFs to aggression (average approach), we used three comparisons: 1) a t-test comparing males that lost fertilizations in their social nest to those that did not, 2) a t-test comparing males that gained at least one EPF to those that did not, and 3) a paired t-test comparing the social sire to the extrapair sire. If the male lost multiple EPFs at his nest, we averaged approach scores of the extrapair sires weighted by the number of chicks they sired. All statistical analyses were conducted in R v3.0.2 (R Core Team, Vienna, Austria).

## RESULTS

### *Paternity Analysis*

Of 217 sampled nestlings, 13 were excluded from paternity assignment, because they were genotyped at 4 or fewer loci ( $n=3$ ), or, because the known mother was genotyped at 4 or fewer loci ( $n=10$ ). All mother-offspring mismatches ( $n=18$ ) occurred at only 1 locus and appeared to be the result of mutation, because 83% of mother-offspring mismatches occurred at *Mme2*, a locus which showed significantly lower heterozygosity than expected ( $p<.05$  after Bonferonni adjustment) and a relatively high frequency of null alleles ( $F_{\text{null}}=0.070$ ). Of the 204 chicks included in the paternity assignment, CERVUS assigned paternity to 68.6% of young ( $n=140$ ) at 95% population-level confidence and 24.5% of young ( $n=50$ ) at 80% confidence. CERVUS did not assign paternity to 6.9% of young ( $n=14$ ) with statistical confidence. We accepted 97% of the strict confidence CERVUS assignments ( $n=136$ ) and 60% of the relaxed confidence assignments ( $n=30$ ). Based on the “total evidence” approach, we rejected 13% of CERVUS assignments ( $n=24$ ), and in these cases, paternity was most often assigned the second most likely sire ( $n=19$ ). Paternity was undefined for the remaining 5 cases. Of the 7% of 207 offspring ( $n=14$ ) to which CERVUS did not assign paternity with statistical confidence, paternity was assigned to the social sire if the social sire mismatched the chick at no more than one locus and matched the social sire better than any other male within 2 territories ( $n=8$ ). Paternity was assigned to an extrapair sire if the

Table 3. Regression analyses relating aggression to annual reproductive success, nest productivity, and nestling growth.

Predictor variable	Coefficient	SE	<i>p</i>
Annual reproductive success	-0.1102	0.1182	0.3575
Nest productivity	-0.02024	0.12200	0.869
Nestling growth rate	-0.21354	0.07903	0.0127

extrapair male was located within two territories of the focal chick, mismatched the chick at no more than 1 locus, and matched the chick better than any other male within two territories (n=5). If these criteria could not be met, paternity was considered undefined (n=1) (Hill et al. 2011). We excluded a total 19 offspring from further analyses, due to missing genetic data, or our inability to assign paternity unequivocally.

#### *Aggression and Reproductive Variables*

Aggression was not significantly related to annual reproductive success in linear regression analysis (coefficient =  $-0.1102 \pm 0.1182$ ,  $F = 0.8681$ ,  $df = 1$  and  $37$ ,  $p = 0.3575$ ,  $R^2=0.023$ ,  $n = 38$ , Table 3). Nor was aggression significantly related to nest productivity (coefficient =  $-0.02024 \pm 0.12200$ ,  $F = 0.0275$ ,  $df = 1$  and  $39$ ,  $p = 0.869$ ,  $R^2=0.001$ ,  $n = 40$ , Table 3). Aggression showed a significant linear relationship with nestling growth rate (coefficient =  $-0.21354 \pm 0.17903$ ,  $F = 7.301$ ,  $df = 1$  and  $23$ ,  $p$

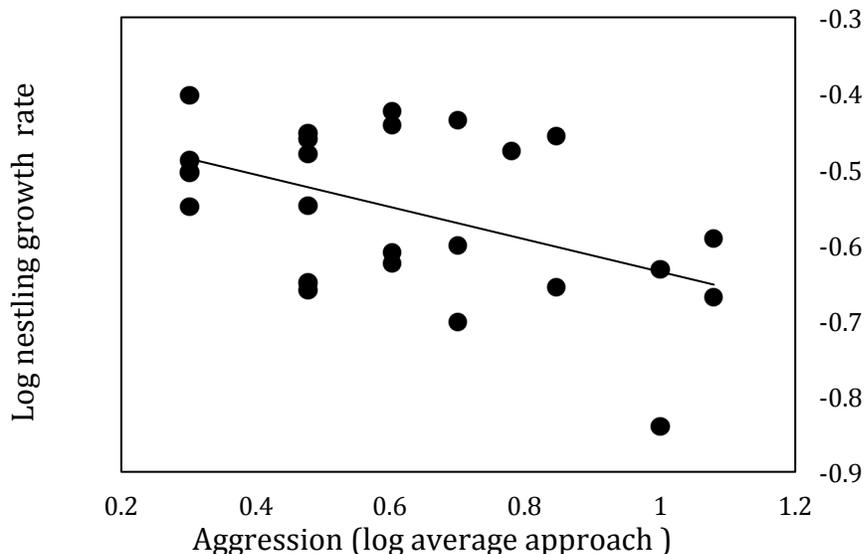


Figure 1. Growth rate has a positive linear relationship with aggression ( $P=0.01$ ). Aggressive males are defined by a low average approach (m), as average approach to the speaker during a playback experiment predicts the likelihood a male will attack an intruder (Searcy et al. 2006).

=0.01272,  $R^2=0.241$ ,  $n=24$ , Table 3, Figure 1), with more aggressive males having young with faster growth rates. Date of nest sampling was unrelated to nestling growth in a multiple regression analysis with aggression (coefficient= $-.0013 \pm 0.001$ ,  $p=0.12198$ ,  $F=5.5169$ ,  $df=2$  and  $22$ ,  $p=0.1418$   $R^2=0.3208$ ,  $n=23$ ).

#### *Characterizing Extrapair Paternity*

In total, sires were assigned to 207 offspring. WPFs were responsible for 86% ( $n=189$ ) of offspring, and EPFs were responsible for 14% of offspring ( $n=27$ ).

We identified 73 to which we were able to assign paternity. Of these 73, 19% ( $n=14$ ) had at least one chick that resulted from an EPF. Of social males ( $n=52$ ), 92% had at least one successful nest ( $n=48$ ), and 27% lost at least 1 WPF in the nest ( $n=13$ ). Our data did not indicate the likelihood of a male to seek out EPFs related to his likelihood of cuckoldry (Chi-square = 0.1586,  $df=1$ ,  $p=0.6921$ ).

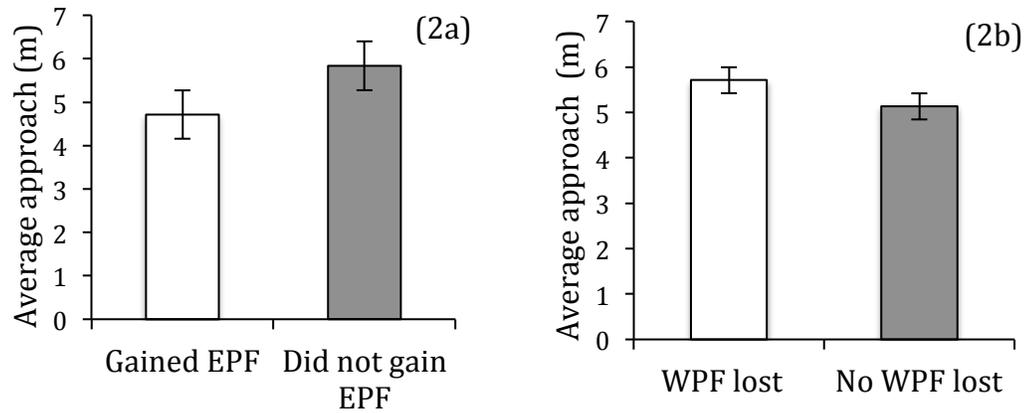


Figure 2. Comparisons between a) males that gained at least one EPF and males that did not gain EPFs, and b) males that lost at least one WPF and males that did not lose WPFs. Error bars indicate  $\pm 1$  standard error of the mean.

### *Aggression and Extrapair Paternity*

We did not see a difference in mean aggression between males that obtained at least one EPF and males that did not ( $t = 1.1396$ ,  $df = 25$ ,  $p = 0.2655$ , Figure 2a). Aggression, also, did not differ between males that were cuckolded and males that were not ( $t = -0.5045$ ,  $df = 23$ ,  $p = 0.6187$ , Figure 2b). In pairwise comparisons, extrapair sires were not more aggressive than their within-pair counterparts ( $t = 0.0901$ ,  $df = 11$ ,  $P = 0.9298$ , Figure 3).

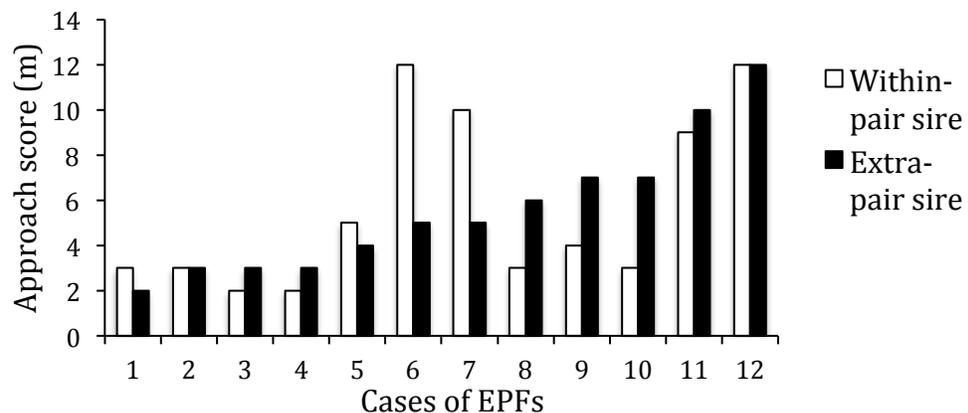


Figure 3. Pairwise comparisons of within-pair (social) sires to extrapair sires.

## DISCUSSION

We did not find evidence that territorial aggression in male song sparrows contributed to annual reproductive success, nest productivity, or patterns of extrapair paternity (Table 3). However, since we only collected data over one breeding season, it may be that our data are confounded by stochastic effects, such as predation and inclement weather, which are the primary causes of nest failure in song sparrows (Arcese et al. 1985). We found a positive relationship between aggression and nestling growth rate (Table 3), which may indicate that aggressive male phenotypes may be favored via selection on their offspring (Arcese and Smith 1985, Duckworth 2006b, Both et al. 1999, Class & Moore 2010, Hegyi et al. 2011).

The reproductive benefits of aggressive, competitive phenotypes to territorial songbirds are well described (eg. Arcese & Smith 1985, Duckworth 2006a, Kunc et al. 2006, Scales et al. 2013). In nightingales, *Luscinia megarhynchos*, song matching, a commonly used signal of aggression, showed a positive relationship with pairing success (Kunc et al. 2006). In Western bluebirds *Sialia mexicana*, aggressive males were more effective at competing for high quality territories (Duckworth 2006a). If aggressive individuals have greater reproductive success, evolutionary theory predicts that individual variation in aggression in a population should decrease over time (Coleman & Wilson 1998), yet territorial aggression shows a great deal of individual variation (Nowicki et al. 2002, Tuttle 2003, Hyman et al. 2004, Sih et al. 2004, Dingemanse et al. 2004, Schuett et al. 2010). In song sparrows, males show consistent individual variation in aggressive responses to playback experiments when measured more than once within the breeding

season (Nowicki et al. 2002, Hyman et al. 2004) or across years (J. Hyman, unpublished data). Consistent individual variation in behavioral responses may indicate an underlying genetic mechanism to the behavioral phenotype, because, in other species aggression has shown the same level of heritability as life history traits (Reale et al. 2007).

Although 14% of sampled chicks were the result of EPFs, the actual percentage of EPFs for the population was likely higher. We were unable to sample all extrapair fertilizations, because our population was not insular, and thus, we likely missed EPFs by males that held territories towards the periphery of the study site. We found no evidence that males that sought extrapair fertilizations were more susceptible to cuckoldry. Nor did our results indicate that males in our population that did not seek EPFs were less susceptible to cuckoldry. Our data did not support a trade-off between mate guarding and seeking EPFs. Game theory modeling predicts that the intensity of mate guarding significantly relates to patterns of paternity (Kokko & Morrell 2005). A trade-off between mate guarding and seeking EPFs has been seen in black-throated blue warblers, *Dendroica caerulescens* (Chuang-Dobbs et al. 2001), bluethroats, *Luscinia svecica* (Johnsen et al. 1998), and purple martins, *Progne subis*, (Wagner et al. 1996), although this relationship is not the rule. Our results are congruent with a prior study on extrapair paternity in song sparrows (Hill et al. 2011), which did not identify a propensity for males to either both gain EPF and lose WPFs, or to neither gain nor lose WPFs. That we did not identify a relationship between mate guarding and risk of cuckoldry may be due to the possibility of complex and non-linear effects of mate guarding on patterns of paternity. If female infidelity increases, game theory predicts that males should increase mate guarding to decrease the risk of cuckoldry. However, if at the same time, females

are capable of circumventing mate guarding and increasing their rate of infidelity, males' incentive to mate guard decreases and the incentive to attempt EPFs increases. To further identify potential trade-offs of mate guarding in song sparrows, we suggest measuring time spent mate guarding and taking into account the possibility of feedback on patterns of paternity.

We found a significant positive relationship between aggression and nestling growth rate (Figure 1) such that the social nestlings of aggressive males grow at a faster rate than the nestlings of unaggressive males. These results do not support a compromise between energy expenditure on parental care versus territorial aggression (Hegner & Wingfield 1987). Two independent studies on Emberizid passerines, dark-eyed juncos, *Junco hyemalis* (Ketterson et al. 1992) and rufous-collared sparrows, *Zonotrichia capensis* (Class & Moore 2010), revealed that male parental investment is directly related to female provisioning rate, in such a way that the feeding rate of the female is inversely proportional to the feeding rate of the male. However, male provisioning was still directly related to nestling body size (Class & Moore 2010).

Nestling growth rates have been shown to affect both the recruitment and reproductive success of offspring (Arcese and Smith 1985, Duckworth 2006b, Both et al. 1999, Class & Moore 2010, Hegyi et al. 2011). Thus, aggressive male song sparrows could achieve higher lifetime reproductive success by producing more successful offspring, even if aggressive males do not produce more total offspring. Aggressive phenotypes may be favored via selection that acts well after fledging. In this study, it remains unknown if nestling growth rate is a result of good parental care, by either the male or the female parent, or a result of 'good genes'. A functional approach, examining

provisioning rates in males and potential compensation by the female, or an experimental approach, swapping nestlings between aggressive and unaggressive males, could contribute information to the debate over the function of extreme phenotypic expression in birds.

We found support for the hypothesis that aggressive phenotypes are favored in song sparrows, because more aggressive males had nestlings that grew faster. These results expand upon the findings of Scales et al. (2013), which suggest reproductive benefits to aggressive phenotypes, because aggressive males in a rural population of song sparrows obtained territories with historically larger clutch sizes than unaggressive males. Due to variation in food availability and habitat structure, selection may act differently upon aggressive phenotypes in urban and rural populations. Both our study and Scales et al. (2013) found evidence of benefits to aggression and no evidence for trade-offs related to aggression. The question remains as to why individual variation in aggression persists in populations of song sparrows; however, further multi-year population level studies can help us to identify how selection and constraints act to shape behavioral variation.

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