3-2014

Nestling Sex Ratios Do Not Support Long-Term Parity In Two Species With Different Life-History Strategies

Noah G. Perlut
University of New England, nperlut@une.edu

Steven E. Travis
University of New England, stravis@une.edu

Catherine A. Dunbar
University of New England

Allan M. Strong

Derek M. Wright
The University of New England

Follow this and additional works at: http://dune.une.edu/env_facpubs

Part of the Environmental Health and Protection Commons, Environmental Indicators and Impact Assessment Commons, Environmental Monitoring Commons, and the Natural Resources Management and Policy Commons

RESEARCH ARTICLE

Nestling sex ratios do not support long-term parity in two species with different life-history strategies

Noah G. Perlut,[1]* Steven E. Travis,[2] Catherine A. Dunbar,[1] Allan M. Strong,[3] and Derek M. Wright[1]

[1] University of New England, Department of Environmental Studies, Biddeford, Maine, USA
[2] University of New England, Department of Biology, Biddeford, Maine, USA
[3] University of Vermont, The Rubenstein School of Environment and Natural Resources, Burlington, Vermont, USA
* Corresponding author: nperlut@une.edu

ABSTRACT

To maximize fitness, breeding adults may respond to environmental processes by adjusting their progeny's sex ratios. R. A. Fisher in 1930 hypothesized that frequency-dependent selection would result in equal investment in sons and daughters over the long term, yielding a balanced sex ratio if the costs of raising a son and daughter are equal. Diverse hypotheses have tried to explain population and brood-by-brood deviations from this mean as well as annual variation by focusing on adult sex ratios, resources, abiotic conditions, and female and male quality. We collected data in 2002–2010 to explore population-level variation in nestling sex ratios in 2 migratory grassland songbird species: the Bobolink (*Dolichonyx oryzivorus*) and Savannah Sparrow (*Passerculus sandwichensis*). These species differ in migratory strategy (long-distance vs. short-distance), and morphological dimorphism. Fisher's hypothesis was rejected for Savannah Sparrows ($n = 684$ nestlings; 39% male) but not rejected for Bobolinks ($n = 390$ nestlings; 53.8% male). No relationship was found between nestling and adult sex ratios measured in the same year. In descriptive analyses at the brood level, male and female body size and age, and ecological conditions (temperature and precipitation) failed to predict nestling sex ratios. Although male nestlings were heavier than female nestlings and resource availability changed through the season, these factors did not influence sex ratios relative to female body size or seasonality. For Savannah Sparrows, larger broods tended to be male-biased. While we were otherwise not able to explain deviation in offspring sex ratio for Savannah Sparrows, our results suggest that the ecological and evolutionary pressures that affect sex ratios may be both species- and population-specific.

Keywords: Bobolink, *Dolichonyx oryzivorus*, Fisher's hypothesis, homeostasis hypothesis, nestling sex ratio adjustment, *Passerculus sandwichensis*, Savannah Sparrow, Vermont

La proporción sexual de los pichones no apoya la igualdad de largo plazo en dos especies con diferentes estrategias de historia de vida

RESUMEN

Para maximizar su aptitud, los adultos que se reproducen podrían responder a procesos ambientales mediante el ajuste de la proporción de sexos en su progenie. Fischer formuló la hipótesis de que la selección dependiente de la frecuencia podría conducir a una inversión similar en hijos e hijas en el largo plazo, produciendo una proporción de sexos balanceada si los costos de criar hijos e hijas son los mismos. Varias hipótesis han tratado de explicar las desviaciones de esta media a nivel de poblaciones y nidadas, así como su variación anual enfocándose en la proporción sexual de los adultos, los recursos, las condiciones abióticas y la calidad de hembras y machos. Recolectamos datos entre 2002 y 2010 para explorar la variación a nivel poblacional en la proporción sexual de pichones de dos aves migratorias de pastizal: *Dolichonyx oryzivorus* y *Passerculus sandwichensis*. Estas especies difieren en su estrategia migratoria (larga vs. corta distancia) y en el dimorfismo en morfología. La hipótesis de Fischer fue rechazada para *P. sandwichensis* ($n = 684$ pichones; 39% machos), pero no para *D. oryzivorus* ($n = 390$ pichones; 53.8% machos). No se encontró relación entre polluelos y adultos en las proporciones sexuales medidas en el mismo año. En análisis descriptivos al nivel de nidadas, la proporción sexual de los pichones no pudo ser explicada por el tamaño corporal ni la edad de machos y hembras, ni por las condiciones ecológicas (temperatura y precipitación). Aunque los pichones macho fueron más pesados que las hembras y la disponibilidad de recursos cambió durante la temporada, estos factores no tuvieron influencia sobre la proporción sexual en relación con el tamaño corporal de las hembras o la estacionalidad. En *P. sandwichensis*, las nidadas más grandes tendieron estar sesgadas hacia incluir más machos. Aunque no pudimos explicar la desviación en la proporción sexual de la descendencia en *P. sandwichensis*, nuestros resultados sugieren que las presiones evolutivas y ecológicas que afectan las proporciones sexual podrían ser específicas de cada especie y de cada población.

INTRODUCTION

Offspring sex ratios are often suggested to be affected by both environmental and evolutionary processes, including population density, mating systems, and sexual selection. Breeding adults may respond to these processes by influencing the sex ratio of their young in an effort to maximize adults' long-term fitness. A broad array of taxa are known to adjust offspring sex ratios, including red deer (*Cervus elaphus*; Clutton-Brock et al. 1986), southern elephant seals (*Mirounga leonine*; Arnbom et al. 1994), fig wasps (*Tetrapus costaricensis* and *Blastophaga* spp.; Herre 1985), Atlantic silversides (*Menidia menidia*; Conover and VanVoorhees 1990), and several species of birds (see reviews by Cockburn et al. 2002, Alonso-Alvarez and Velando 2003). Offspring sex ratio adjustment at the individual level may have either positive or negative effects on the population growth rate. For example, an overabundance of males could reduce the effective population size and cause population decline (Clout et al. 2002); however, where selection processes are more variable, biased sex ratios could support population growth (Herre 1987).

Fisher (1930) proposed that frequency-dependent selection would result in an equal investment in sons and daughters over the long term, leading to a balanced sex ratio if the costs of raising a son and daughter are equal. The homeostasis hypothesis, a close extension of Fisher's (1930) classic hypothesis, suggests that long-term parity is maintained when females produce more of the sex that is rarer in the adult population (Creel and Creel 1997) as a form of negative frequency-dependent selection; however, Donald (2007) reviewed 173 bird species and found that 65% of adult sex ratios were biased (typically favoring males), while in 114 species only 16% of studies showed nestling sex ratios bias. This pattern is contrary to the homeostasis hypothesis, assuming that studies had similar power to detect nestling sex ratio biases as they did to detect adult sex ratio biases. These results suggest that the factors that influence sex ratio adjustment—if they are actually adjusted—vary between species and populations (Donald 2007).

Here, we tested the Fisher (1930) and homeostasis hypotheses to explore population-level nestling sex ratios in 2 migratory grassland songbird species, Bobolinks (*Dolichonyx oryzivorus*) and Savannah Sparrows (*Passerculus sandwichensis*), breeding in the same agricultural habitats. These species use similar sites for breeding and food resources, comprise >92% of all obligate grassland songbirds breeding in our study region (Shustack 2004), and >99% of the birds breeding in our study fields. Both species and both sexes show high natal fidelity to our study sites (94% of adults and 30% of juveniles; Fajardo et al. 2009); therefore, these recruits have the potential to collect information on the breeding sex ratios of the year they were born and current year, creating an environment where they could adjust their sex ratio output accordingly. Likewise, adult Bobolinks and Savannah Sparrows show high apparent survival rates in this study system (Perlut et al. 2008a), providing them with an opportunity to assess the current year's adult sex ratio with past years' and adjust their efforts accordingly. They differ with respect to migratory behavior, which affects how much reproductive effort they expend (Perlut et al. 2006).

We present a long-term study of 2 species with different life history strategies, allowing us to better understand variation in sex ratios and therefore population-level processes. We predicted that Savannah Sparrows, whose shorter migration allows a greater opportunity to nest multiple times, would show greater within-year variation in brood sex ratios because ecological and social pressures vary across the breeding season (which can be up to 5 weeks longer than Bobolinks); therefore, in Savannah Sparrows, multiple factors may influence sex ratio adjustment as the conditions vary among individuals, environments, and years.

After testing the Fisher and homeostasis hypotheses in response to these species' known ecology and natural history as well as what is known for other species, we conducted descriptive analyses to better understand nest-level variation. These species differ in their degree of sexual dimorphism, with Bobolinks showing greater morphological dimorphism than Savannah Sparrows; Bobolink and Savannah Sparrow males are 20% and 5%, respectively, larger than females (Martin and Gavin 1995, Wheelwright and Rising 2008). We therefore predicted that if Bobolinks showed a female-biased sex ratio, these adjustments may reflect the increased costs of raising one sex over the other, where investment in parental care may be greater for sons than for daughters (male nestlings are heavier than female nestlings by the end of parental care; see Results). We explored correlations between female body size and age with sex ratios (Dowling and Mulder 2006). Similarly, we tested if male structural size (Yamaguchi et al. 2004) or true paternity influenced sex ratios. We then tested if there were differences among brood sizes and in mass between male and female nestlings because the larger sex may be at a disadvantage in times of lower resources (e.g., early in the breeding season; Trivers and Willard 1973). Finally, we tested if ecological conditions, particularly temperature and precipitation during the laying female's fertile period, affected sex ratios (Saino et al. 2008).

METHODS

Study Area

Our work took place in the Champlain Valley of Vermont and New York, USA, which includes 146,000 ha of managed grasslands (NASS 2010). We sampled 6 hayfields and 3 pastures in Hinesburg and Shelburne, Vermont. Field sizes ranged from 13.2 to 38.3 ha (mean 21.1 ha; see Perlut et al. 2006 for additional details on vegetation).

Study Species

Bobolinks and Savannah Sparrows are grassland obligate, ground-nesting songbirds whose breeding distribution includes diverse grassland habitats across northern North America (Martin and Gavin 1995, Wheelwright and Rising 2008). In our study region there is little variation between species in breeding habitats, clutch size (Perlut et al. 2006), nest location (Perkins et al. 2013), or food resources fed to nestlings (A. Strong personal communication). In addition, both species are socially and genetically polygynous, where males can have multiple female social mates, and multiple males can have paternity in a single brood (Gavin and Bollinger 1985, Perlut et al. 2008b). By contrast, Bobolinks arrive in Vermont in mid- to late-May, typically raise only one brood per summer, and are highly dimorphic in body size (males are up to 20% larger; Martin and Gavin 1995), whereas Savannah Sparrows arrive in Vermont in late-April, can raise multiple broods, and males are approximately 5% larger than females (Wheelwright and Rising 2008).

Field Sampling

We collected reproductive and behavioral data for Bobolinks in 2002–2005, 2007–2008, and 2010, and for Savannah Sparrows in 2002–2010 from early-May to late-July each year. Nests were located through behavioral observations. Female and male nest association was identified by incubation (female only), provisioning, and territory defense behavior. Nests were visited every 1–2 days to assess their status until fledging or failure. We collected location data for each nest using a hand-held GPS unit. Adults were captured with mist nets, banded with 3 colored and a single U.S. Geological Survey band. We collected a small (20–60 μL) sample of blood and took standard morphological measurements: wing length, tarsus, bill length, bill width, and bill depth. Nestlings were banded, weighed, and blood samples were collected at approximately 6 days of age (86% of nestlings were sampled between days 5 and 7; because of the timing of when we found the nest, or to minimize disturbance at the nest, <1% were sampled on days 3 and 10). For both adults and nestlings, blood was placed on Whatman filter paper and frozen at −80°C until analyzed.

To evaluate the year-specific adult sex ratio, we spent the first 1–2 weeks of each season blanket netting each study field, attempting to catch each breeding adult. We continued our banding efforts as we found nests through the season. If a bird was socially associated with a nest but was not banded, we caught it at the nest site. Because we attempted to color band every adult, we were able to determine adult sex ratios through annual banding records, parental care nest association data, and comprehensive resighting data that we maintained throughout each breeding season. These data were collected on each field every other day for as long as nests were active on a given field. A bird was included within the adult sex ratio if it met at least one of the following criteria: it was identified as socially associated with a nest, was color banded in a previous year and resighted (or recaptured) on the same field, or was banded within the given year and resighted at least one time on the field at least 1 week after banding.

These combined efforts also allowed us to age individual birds by site residency (age cannot be assessed by plumage in these species; Pyle 1997). A new (unbanded) bird was assumed to be a 2-year-old recruit. In this population, both species show extremely high breeding (and natal) site fidelity; 94% of all individuals who survive to the following year (regardless of the habitat they used or reproductive success during the previous year) returned to the same field they were in previously (Fajardo et al. 2009). Each year, from our resight, banding, and nest records, unbanded birds accounted for <3% of all territorial birds on our study fields.

Molecular Analysis of Sex Ratios

DNA was extracted from blood samples using a Qiagen DNeasy Kit and then underwent polymerase-chain-reaction (PCR) amplification of sex-specific DNA markers using primers described by Han et al. (2009). Gels were visualized and photographed under UV illumination and scored by eye. Males could be identified by single (Z-linked) bands whereas females were identified by double (Z- and W-linked) bands. Each gel included one adult male and female as a reference.

We restricted our analysis to only those nests that were fully sampled (i.e. blood was collected from nests in which the number of nestlings was equal to the number of eggs laid; 54.7% and 48.3% of all broods for Bobolinks and Savannah Sparrows, respectively). Thus, we removed any nests that had natural brood reduction or nests where we were unable to amplify DNA from certain individuals. Due to the binomial nature of sex ratio data, where possible we adopted a generalized linear modeling (GLM) approach and analyzed sex ratios as numbers of males and females within broods (Wilson and Hardy 2002). In situations where this was not possible (e.g., homeostasis hypothesis, where we tested the sex ratio of a population), sex ratios

were calculated as percent males within broods and arc-sin transformed to adjust for non-normality.

Statistical Analysis

After initially testing for long-term parity and its possible dependence on year within populations of both Bobolinks and Savannah Sparrows, we then tested if adult sex ratios from both the present and previous year explained variation in sex ratios. We examined the correlation between nestling sex ratios and female morphology and age, male morphology, the total number of offspring males sired, and proportion of offspring produced through extra-pair paternity (Savannah Sparrows only because paternity data for Bobolinks was unavailable). We also examined environmental variables including seasonality (clutch completion date), average high temperature, average low temperature, and total precipitation beginning 5 days before the first egg was laid until the last egg was laid, spanning the beginning to the end of the fertile period (Kempenaers 1993); sex is determined 1–2 hr before ovulation (Rutkowska and Badyaev 2008). We also compared the mass of male versus female nestlings to determine if one sex was likely to require a greater parental investment than the other. All measures of female morphology, including tarsus length, wing cord, and bill volume, were converted to Z-scores (all measurements were collected by one observer, N. Perlut).

To address male paternity in Savannah Sparrows we determined parentage for nests found in 2002–2006. Paternity analysis was performed with 4 hypervariable microsatellite loci: $Psa12$ (Freeman-Gallant et al. 2005); $Escu6$ (Hanotte et al. 1994); and $Mme1$ and $Mme8$ (Jeffery et al. 2001). All molecular and paternity assignment methods followed Freeman-Gallant et al. (2005) and Perlut et al. (2008b). This population showed high allele diversity (range 10–38) and a 0.91547 probability of exclusion (see Perlut et al. 2012 for additional information on exclusion probabilities). We assigned paternity to all nestlings in 44 broods. All offspring matched their mothers at all loci; 35 of 44 broods had at least one extra-pair young; 18 of 44 broods had both extra-pair and within-pair young. We identified the genetic father in 82% of nestlings.

We tested for parity separately in Bobolinks and Savannah Sparrows by combining data over all sampling years and using nests (i.e. individual broods) as the sampling units. Initially, we constructed a logistic regression model (a form of GLM appropriate to binomially distributed data such as brood-specific sex ratios; Wilson and Hardy 2002) for each species to determine if brood sex ratios varied by year. These GLM models, which provided a means of applying different weights to individual broods depending on the number of nestlings present by binding together the actual number of males and females within broods as a single response variable, were implemented

with binomial errors and the logit-link function using R version 2.15.1 (R Development Core Team 2010). We evaluated the overall effect of year on brood sex ratios by comparing each year-based model with a null model (with year removed) via F-tests, as recommended by Wilson and Hardy (2002). Once we had confirmed that sex ratios did not vary significantly from year to year in either species, we proceeded to test for parity within each species using broods from all years combined. These combined tests were run according to Neuhäuser (2004), who developed a Z-test for testing the null hypothesis of parity in sex-ratio studies, which explicitly adjusts for the non-independence of nestlings within broods.

Postma et al. 2011 suggested that for adjustment in sex ratios to be possible there must be extra-binomial variance in sex ratios (i.e. there must be more male- and/or female-biased broods than expected by chance); therefore, we conducted an extra-binomial variance test according to the methods of James (1975). As described in Krackow et al. (2002), this method is appropriate when many small broods of unequal size are available for analysis and relies on a one-tailed Z-test for detecting significant positive deviations from expected variance. We conducted separate tests for Bobolinks and Savannah Sparrows, combining broods over all sampling years within species.

To test the homeostasis hypothesis (that population sex ratios remain near parity because annual nestling sex ratios are adjusted to the current or the previous year's adult sex ratios), we ran separate simple linear regressions by species with arc-sin transformed nestling sex ratio (percent males) as the response variable, and arc-sin transformed adult sex ratios as the explanatory variables.

We tested the female adult morphology and ecological factors employing a forward model selection process based on a series of logistic regression models within each species. We chose this descriptive approach because, to our knowledge, there are no published data identifying what characters define fitness in male or female Bobolinks or Savannah Sparrows. Initially, each model treated brood sex ratio as a function of a single explanatory variable, where the explanatory variables included all female morphological and environmental variables as well as female age. Because of the multiple testing required by this approach (9 single-factor logistic regressions per species), we used a Bonferroni-adjusted test-wise α-level of 0.0056 for each individual test. All explanatory variables yielding significant p-values were ranked, and the variable returning the lowest p-value was subsequently combined with all other explanatory variables in a series of 2-way logistic regression models. All 2-way models with significant effects beyond the original main effect were ranked again according to their overall residual deviances, and the selection process continued to test a series of 3-way models, and so on, with the goal of ultimately arriving at a
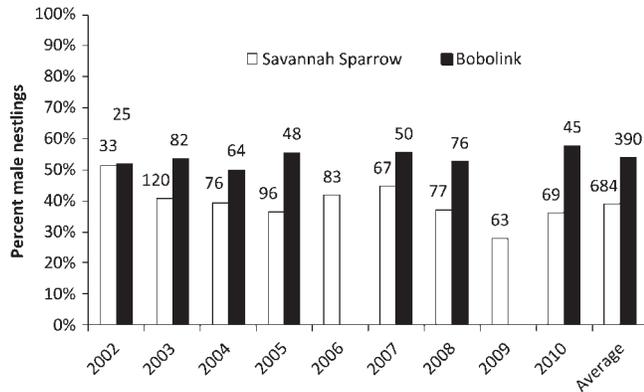
FIGURE 1. Percentage of male Bobolink and Savannah Sparrow nestlings across years. No significant variation was found. Sex ratios for Bobolinks in 2006 and 2009 were not evaluated. Values indicate number of nestlings sampled.

model retaining only significant terms. Note that year was also introduced as an explanatory variable at the level of the 2-way and higher-order models to allow the possibility of significant female morphological and environmental effects that were variable across years. As above, F-tests were used to determine whether explanatory variables and their interactions were adding significant explanatory power to the overall model, treating brood sex ratio as the response variable by binding together the actual number of males and females within broods.

We tested for effects of male morphology on Savannah Sparrows using a logistic regression approach similar to that described for females. In the case of males, the forward selection process began with a group of centered variables (Z-score), which included tarsus length, wing cord length, bill volume, and mass, as well as the total number of offspring sired, and proportion of offspring produced through extra-pair paternity. Because many males were sampled in multiple years, which created an issue of non-independence across years, we first ran our logistic regression models separately by year for each year that paternity data were available (2002–2005). Because of multiple testing within years (6 single-factor logistic regressions per year), test-wise α-levels were Bonferroni-adjusted to 0.0083. Subsequently, we ran the models on all years combined but using each male only once, the first time he appeared among the samples. In several instances, this procedure resulted in models with significant interactions and no significant main effects. Because of the difficulty of biologically interpreting such models, we herein only report interactive models with significant main effects.

Finally, we ran separate ANCOVAs by species, treating the mass of nestlings as the response variable, sex as the grouping variable, and nestling age (in days) as the covariate, to compare nestling mass adjusted for age

between males and females. If male nestlings were found to be larger in either species, we then determined whether broods with more males were more likely to fail (for the period between blood sampling and fledging) by running a logistic regression of nest fate (fail or fledge) on sex ratio. Nest fate, as a binary variable, is appropriately modeled with binomial errors and the logit-link function. Sex ratio, which served as the explanatory variable in this case, was entered into the model as the arc-sin transformed nestling sex ratio (percent males). Year (and sex-ratio by year) was also included as a factor in each species' model to allow for variability in the effect of sex ratio on nest fate by year. We used a logistic-regression model for testing the effects of brood size on sex ratio.

We transformed each of the explanatory variables as necessary to meet the assumptions of the various tests we employed. Because they represented counts (in units of years), female ages were square-root transformed prior to analysis. Because of significant skewness, precipitation was log-transformed. To simplify interpretation in logistic regressions that included multiple explanatory variables, all variables were centered (by subtracting the mean from all individual measurements) prior to analysis (see Quinn and Keough 2002). We considered all nonsignificant p-values evidence of poor model fit. All statistical analyses were performed in R, version 2.15.1 (R Development Core Team 2010).

RESULTS

Population-Level Hypotheses
We sampled 82 Bobolink (390 nestlings) and 181 Savannah Sparrow (684 nestlings) broods in which we obtained DNA from every egg laid. After determining that there was no difference in annual brood sex ratios across years (Bobolink: $F = 0.158$, df = 6 and 80, $P = 0.987$; Savannah Sparrow: $F = 1.016$, df = 8 and 179, $P = 0.426$), we combined broods over all years within each species. Over all years combined, Savannah Sparrows showed a significant female bias ($Z = -5.878$, $P < 0.001$), significantly deviating from the Fisher hypothesis of parity, with just 39% of nestlings sexed as male. In comparison, the Bobolink nestling population did not differ significantly from parity ($Z = 1.523$, $P = 0.064$; Figure 1), although there was a slightly higher proportion of males among the nestlings (53%).

Significant extra-binomial variance was detected for Savannah Sparrows but not for Bobolinks. For Bobolinks, the number of broods may have simply provided insufficient power for detecting extra-binomial variance because the P-value was relatively small but nonsignificant ($P = 0.075$, $Z = 1.437$). For Savannah Sparrows, extra-binomial variance was indicative of overdispersion, with a greater sex-bias among broods than expected by chance (Z
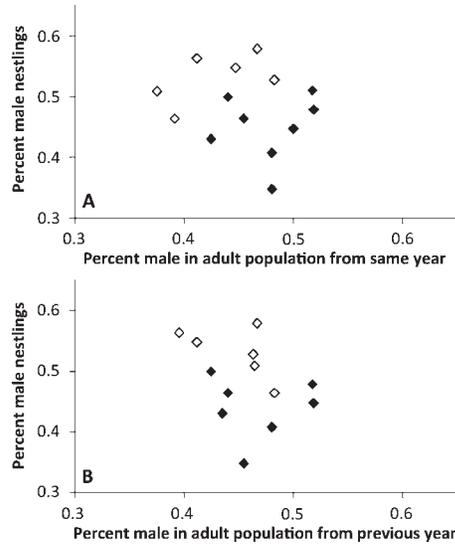
FIGURE 2. Nestling and adult sex ratios (homeostasis hypothesis) measured in the same year (**A**) or previous year (**B**) for Bobolinks (open diamonds) or Savannah Sparrows (filled diamonds) breeding in the Champlain Valley of Vermont. No relationships were found.

$= 1.651$, $P = 0.049$). Although both species showed more adult females in the population, sex ratios did not deviate from parity for either species (Bobolink: $G^2 = 3.46$, df $= 1$, $P = 0.063$, mean percent male 0.45 [min 0.40, max 0.48]; Savannah Sparrow: $G^2 = 0.732$, df $= 1$, $P = 0.39$, mean percent male 0.47 [min 0.42, max 0.52]). Adult sex ratios were considered consistent across years because there was no significant interaction between sex and year for either

species (Bobolink: $G^2 = 1.57$, df $= 8$, $P = 0.99$; Savannah Sparrow: $G^2 = 1.12$, df $= 8$, $P = 0.99$).

To determine how long-term parity is maintained over time, we tested the homeostasis hypothesis. No relationship was found between nestling and adult sex ratios measured in the same year (Bobolink: $F = 4.373$, df $= 1$ and 5, $P = 0.091$; Savannah Sparrow: $F < 0.001$, df $= 1$ and 5, $P = 0.988$; Figure 2) or from adult sex ratios of the previous year (Bobolink: $F = 4.358$, df $= 1$ and 5, $P = 0.091$; Savannah Sparrow: $F < 0.001$, df $= 1$ and 5, $P = 0.978$).

Nest-Level Sex Ratio Descriptive Analyses

No aspect of female morphology explained variation in nestling sex ratios; for Bobolinks, the unadjusted P-values from the 3 single-term logistic regression models run on female morphological variables ranged from 0.067 to 0.349; for Savannah Sparrows, P-values ranged from 0.450 to 0.957 (Table 1). The distribution of female ages for Bobolinks and Savannah Sparrows, respectively, were: 2-yr-old 68% and 56%; 3-yr-old 14% and 25%; 4-yr-old 10% and 13%; 5-yr-old 6% and 4%; 6-yr-old 0% and 1%; 7-yr-old 1% and 1%; and 8-yr-old 1% and 0%. Female age also did not affect offspring sex ratios (Bobolinks: $P = 0.712$; Savannah Sparrows: $P = 0.309$; Table 1). No ecological factors, including seasonality, average high temperature, low temperature, or precipitation during egg laying, related to nestling sex ratios (Bobolinks: P-values 0.600–0.952; Savannah Sparrows: P-values 0.197–0.946; Table 1).

Similarly, male body size and paternity did not explain nestling sex ratios. For male Savannah Sparrows across all years, male mass, tarsus length, wing length, bill volume, extra-pair paternity, and total paternity did not explain

TABLE 1. *F*-test results from one-way logistic regression models run on all female morphological and environmental variables for Bobolinks and Savannah Sparrows breeding in the Champlain Valley of Vermont.

Species	Variable tested	F	P
Bobolink	Tarsus length	$F = 0.888$, df $= 1, 76$	0.349
	Wing cord length	$F = 2.632$, df $= 1, 64$	0.105
	Bill volume	$F = 3.443$, df $= 1, 76$	0.067
	Age	$F = 0.137$, df $= 1, 71$	0.711
	Clutch completion date	$F = 0.277$, df $= 1, 80$	0.599
	Distance to field edge	$F = 0.029$, df $= 1, 78$	0.865
	Mean high temperature	$F = 0.004$, df $= 1, 80$	0.948
	Mean low temperature	$F = 0.017$, df $= 1, 80$	0.898
	Total precipitation	$F = 0.004$, df $= 1, 80$	0.952
Savannah Sparrow	Tarsus length	$F = 0.003$, df $= 1, 128$	0.957
	Wing cord length	$F = 0.573$, df $= 1, 131$	0.450
	Bill volume	$F = 0.469$, df $= 1, 126$	0.495
	Age	$F = 1.039$, df $= 1, 177$	0.309
	Clutch completion date	$F = 0.005$, df $= 1, 168$	0.946
	Distance to field edge	$F = 1.678$, df $= 1, 175$	0.197
	Mean high temperature	$F = 0.722$, df $= 1, 163$	0.397
	Mean low temperature	$F = 0.850$, df $= 1, 163$	0.358
	Total precipitation	$F = 0.022$, df $= 1, 163$	0.882

TABLE 2. *F*-test results from one-way logistic regression models run on all male morphological and paternity variables in Savannah Sparrows breeding in the Champlain Valley of Vermont.

Year	Variable tested	F	P
2002	Tarsus length	$F = 0.122$, df = 1, 6	0.739
	Wing cord length	$F = 1.685$, df = 1, 5	0.251
	Bill volume	$F = 0.016$, df = 1, 6	0.902
	Mass	$F = 0.854$, df = 1, 5	0.398
	Total paternity	$F = 0.308$, df = 1, 6	0.599
	Percent Extra-pair paternity	$F = 0.226$, df = 1, 6	0.634
2003	Tarsus length	$F = 0.340$, df = 1, 22	0.566
	Wing cord length	$F = 0.254$, df = 1, 15	0.622
	Bill volume	$F = 0.001$, df = 1, 22	0.979
	Mass	$F = 4.262$, df = 1, 14	0.058
	Total paternity	$F = 0.439$, df = 1, 22	0.514
	Percent Extra-pair paternity	$F = 0.002$, df = 1, 22	0.969
2004	Tarsus length	$F = 3.056$, df = 1, 31	0.090
	Wing cord length	$F = 1.173$, df = 1, 12	0.300
	Bill volume	$F = 0.017$, df = 1, 31	0.899
	Mass	$F = 0.162$, df = 1, 11	0.695
	Total paternity	$F = 2.035$, df = 1, 31	0.164
	Percent Extra-pair paternity	$F = 1.910$, df = 1, 31	0.177
2005	Tarsus length	$F < 0.001$, df = 1, 10	0.984
	Wing cord length	$F = 0.452$, df = 1, 9	0.518
	Bill volume	$F = 0.966$, df = 1, 10	0.349
	Mass	$F = 5.499$, df = 1, 9	0.044
	Total paternity	$F = 0.011$, df = 1, 10	0.920
	Percent Extra-pair paternity	$F = 0.053$, df = 1, 10	0.822
All Years	Tarsus length	$F = 0.757$, df = 1, 48	0.390
	Wing cord length	$F = 0.015$, df = 1, 26	0.904
	Bill volume	$F = 0.115$, df = 1, 48	0.736
	Mass	$F = 0.524$, df = 1, 24	0.476
	Total paternity	$F = 0.107$, df = 1, 48	0.745
	Percent Extra-pair paternity	$F = 0.003$, df = 1, 48	0.955

variation in nestling sex ratios (*P*-values 0.390–0.955; Table 2).

For Bobolinks, there was no effect of brood size on nestling sex ratio ($G^2 = 0.34$, df = 1, $P = 0.56$); however, for Savannah Sparrows larger broods tended to have male-biased sex ratios ($G^2 = 5.50$, df = 1, $P = 0.02$; probability male $= -1.6817 + 0.3159$*brood size; Figure 3). Male nestlings weighed more than female nestlings for Bobolinks ($F = 29.94$, df = 1 and 468, $P < 0.001$) and Savannah Sparrows ($F = 11.13$, df = 1 and 726, $P < 0.001$), although the difference was greater for Bobolinks (females: Mass = 8.309 [0.683 SE] + 1.394*Day; males: Mass = 9.711 [0.678 SE] + 1.394*Day) than for Savannah Sparrows (females: Mass = 5.215 [0.394 SE] + 1.059*Day; males: Mass = 5.689 [0.402 SE] + 1.059*Day). However, the nestling sex ratio did not affect nest fate (fledge or fail) for either species (Bobolinks: $F = 0.62$, df = 1 and 67, $P = 0.433$; Savannah Sparrows: $F = 0.003$, df = 1 and 162, $P = 0.954$), and the interaction with year was not significant for either species (Bobolinks: $F = 0.03$, df = 6 and 67, $P = 1.000$; Savannah Sparrows: $F = 1.47$, df = 8 and 162, $P = 0.174$).

DISCUSSION

Overall nestling sex ratios were significantly female-biased over a span of 9 years for Savannah Sparrows but did not differ from parity over 7 years for Bobolinks. A closer look
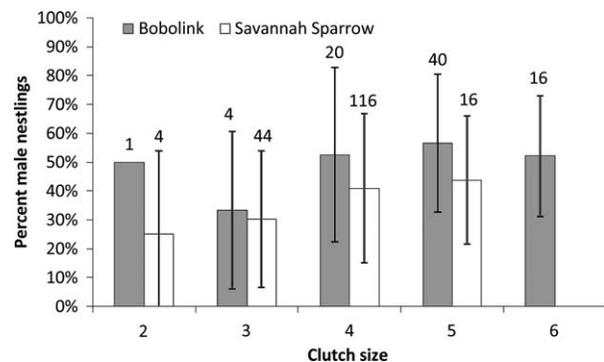
FIGURE 3. Percent male nestlings compared with clutch size for Bobolinks and Savannah Sparrows. Nestling sex ratios were not associated with clutch size; however, for Savannah Sparrows, larger clutches tended to be male-biased. Error bars indicate standard deviation, and values indicate sample size.

at the variation within the data revealed that nestling sex ratios were neither influenced by adult sex ratios in the given or preceding year (i.e. no support for the homeostasis hypothesis) nor did they vary significantly among years. The lack of support for the homeostasis hypothesis generally agrees with the majority of other studies. While nestling sex ratios have been found to be positively related, negatively related, or unrelated to adult sex ratios, most commonly offspring sex ratios have been balanced while adult sex ratios were male-biased (reviewed in Donald 2007). Our results for Bobolinks support Donald (2007) in that the adult population was female-biased and nestling sex ratios were balanced, but conflict for Savannah Sparrows, which showed a female-biased adult sex ratio and female-biased nestling sex ratio (this trend was not predictive on an annual basis because our results did not support the homeostasis hypothesis). In this study system, for both species, male apparent survival is 35% higher than female apparent survival (Perlut et al. 2008a). Given these sex-biased adult survival rates, to maintain stable populations, first-year survival rates would have to be female-biased and/or nestling sex ratios would have to be strongly female-biased.

Contrary to our results, Wheelwright and Seabury (2003) analyzed a 14-year dataset and found 50:50 Savannah Sparrow nestling sex ratios in an island population, and these ratios did not vary among years, timing of the breeding season, or in relation to adult sex ratios. Natal site fidelity was similar between this island (11.2%; Wheelwright and Mauck 1998) and our mainland population (7.5%; N. Perlut personal communication), and adult sex ratios were female-biased on both the island and our mainland population. Saino et al. (2008) studied Barn Swallows (*Hirundo rustica*) with a 10-year dataset and observed differences among years; however, the significant effects explaining sex ratio variation did not vary among years. Bobolinks produce only one brood per year, which may be explained by their long-distance migration to southern South America each year; thus, males are likely to face intense competition for limited mating opportunities, and many males may not mate. Yet, nestling sex ratios were not different from parity in this species. In comparison, Savannah Sparrows can produce multiple broods per year because they have a relatively short migration distance, and they showed strongly female-biased nestling sex ratios.

Although ambient temperatures increased through the breeding season during each year of the study, sex ratios were not affected by the year, seasonality, or the other ecological and environmental conditions that we evaluated. These results contrast with recent studies in which seasonality was a key factor explaining variation in nestling sex ratios (Husby et al. 2006, Graham et al. 2011) but agree with a lack of seasonal sex ratio differences for another grassland species, the Corn Bunting (Miliaria calandra; Hartley et al. 1999) and one cooperative breeder, the Purple-crowned Fairy-wren (*Malurus coronatus*; Kingma et al. 2011). Similarly, in the Aquatic Warbler (*Acrocephalus paludicola*), sex ratios were not affected by seasonality or mean daily temperature; however, simulation modeling suggested that low ambient temperatures during the fertile period led to female-biased nestling sex ratios (Dyrcz et al. 2004). Because sex ratios did not change through the season, females did not invest more in males when resources such as invertebrate prey were most abundant in early- to mid-July (Zalik and Strong 2008), and they did not respond when females could better estimate the adult sex ratio (later in the season; Bensch et al. 1999).

Another potential reason to produce males earlier in the season (particularly because males are larger) is that an earlier fledging date gives them more time to prepare for migration (Dolan et al. 2009). The lack of a seasonal effect in our current study may conflict with what is known for another grassland nesting species, the Skylark (*Alauda arvensis*), where males were favored earlier in the season; Eraud et al. (2006) hypothesized, in considering results of previous studies on their study system, that males were produced during a period of higher resource availability; therefore, given that males in both species are the larger sex, Fisher's hypothesis would have predicted a slightly female-biased sex ratio. Also related to seasonality and food, nest fate in our study was not affected by the sex ratio of the nestlings when resources were scarce. Although male nestlings weighed significantly more than female nestlings (particularly for Bobolinks), suggesting they require more resources, sex ratios did not vary based on the weight of nestlings. We only monitored nestling survival through the fledging period, however, and it is possible that there were differences between sexes in the post-fledging period while still receiving parental care because larger nestlings may have higher rates of post-fledging survival (Naef-Daenzer et al. 2001, Potti et al. 2002). Furthermore, this result may be spurious if nest fate was unrelated to nestling food resource availability.

For Savannah Sparrows only, larger broods tended to be male-biased. Dyrcz et al. (2004) found that for Aquatic Warblers, brood size influenced nestling sex ratio; however, larger broods tended to be female-biased (this species had a similar range of brood size, 2–6, to Savannah Sparrows and Bobolinks). The White-throated Dipper (*Cinclus cinclus*) also showed extreme female-bias for the largest brood size (6); Øigarden and Lifjeld (2013) explained this deviation as the different costs of rearing heavier sons than lighter daughters. Although male Savannah Sparrow nestlings were heavier than female nestlings, our results indicated no costs to rearing the heavier sex.

In addition, no relationship was found between true paternity, extra-pair paternity, or male morphology and sex ratios in Savannah Sparrows. As with our results, Neto et al. (2011) found that Savi's Warblers (*Locustella luscinioides*) also showed female biased nestling sex ratios, but these could not be explained by male size, female size, or extra-pair paternity. To our knowledge, no study has characterized attractiveness in Savannah Sparrows (or in Bobolinks); our result further suggests that in these species the morphological characters that influence reproductive processes are simply not known. Taff et al. (2011) found an interaction between male ornamentation and age influenced nestling sex ratios of the Common Yellowthroat (*Geothlypis trichas*). Savannah Sparrows, however, are monomorphic in plumage and only slightly dimorphic in other aspects of their morphology. Because this species has no known ornament, we did not test any plumage characteristic (although we found no effect of female age on sex ratios for either species). Alternatively, Bobolinks show strong plumage and morphological dimorphism and thus may have been the more appropriate species to test differences among males.

Our results contrast with Fisher's long-term parity hypothesis and the recent studies that show diverse causation of nestling sex ratio manipulation. We expect that other sex ratios studies have found similar results as ours but have not published due to the lack of significant results (Hasselquist and Kempenaers 2002). Furthermore, many of these studies (although not all) used substantially smaller datasets (more prone to Type I errors) and covered fewer years, factors that likely affected our results (Hasselquist and Kempenaers 2002). We sampled nestlings at the point where the population could most reliably and safely be censused (~day 6, minimizing exposure risk to predation). Ideally, every study would also evaluate the sex ratio at both the clutch completion date and the point of independence from parental care, thereby assessing the functional sex ratio for each annual cohort. In the future, we hope to use radio telemetry to assess post-fledgling survival. While we could not explain differences between species, a 2-year study found factors affecting sex ratios to vary between 2 forest-nesting species using the same habitats but with differing foraging strategies (Stauss et al. 2005). We hope this work encourages broader studies in habitats with greater species richness and ecological diversity, exploring these questions with long-term datasets and with multiple species comparisons.

ACKNOWLEDGMENTS

LITERATURE CITED

Alonso-Alvarez, C., and A. Velando (2003). Female body condition and brood sex ratio in Yellow-legged Gulls *Larus cachinnans*. Ibis 145:220–226.

Arnbom, T., M. A. Fedak, and P. Rothery (1994). Offspring sex ratio in relation to female size in southern elephant seals, *Mirounga lionina*. Behavioral Ecology and Sociobiology 35:73–378.

Bensch, S., H. Westerdahl, B. Hansson, and D. Hasselquist (1999). Do females adjust the sex of their offspring in relation to the breeding sex ratio? Journal of Evolutionary Biology 12:1104–1109.

Clout, M. N., G. P. Elliott, and B. C. Robertson (2002). Effects of supplementary feeding on the offspring sex ratio of kakapo: A dilemma for the conservation of a polygynous parrot. Biological Conservation 107:13–18.

Clutton-Brock, T. H., S. D. Albon, and F. E. Guinness. (1986). Great expectations: Maternal dominance, sex rations, and offspring reproductive success in red deer. Animal Behaviour 34:460–471.

Cockburn, A., S. Legge, and M. C. Double (2002). Sex ratios in birds and mammals: Can the hypotheses be disentangled? In Sex Ratios. Concepts and Research Methods (I. C. W. Hardy, Editor). Cambridge University Press, Cambridge. pp. 266–286.

Conover, D. O., and D. A. VanVoorhees (1990). Evolution of a balanced sex ratio by frequency dependent selection in a fish. Science 250:1556–1558.

Creel, S., and N. M. Creel (1997). Lion density and population structure in the Selous game reserve: Evaluation of hunting quotas and offtake. African Journal of Ecology 35:83–93.

Dolan, A. C., M. T. Murphy, L. J. Redmond, and D. Duffield (2009). Maternal characteristics and the production and recruitment of sons in the eastern kingbird. Behavioral Ecology and Sociobiology 63:1527–1537.

Donald, P. F. (2007). Adult sex ratios in wild bird populations. Ibis 149:671–692.

Dowling, D. K., and R. A. Mulder (2006). Combined influence of maternal and paternal quality on sex allocation in Red-capped Robins. Journal of Evolutionary Biology 19:440–449.

Dyrcz, A., H. Sauer-Gürth, E. Tkadlec, and M. Wink (2004). Offspring sex ratio variation in relation to brood size and mortality in a promiscuous species: The Aquatic Warbler *Acrocephalus paludicola*. Ibis 146:269–280.

Eraud, C., J. Lallemand, and H. Lormee (2006). Sex-ratio of Skylark *Alauda arvensis* broods in relation to timing of breeding. Bird Study 53:319–322.

Fajardo, N., A. M. Strong, N. G. Perlut, and N. J. Buckley (2009). Natal and breeding dispersal of Bobolinks and Savannah Sparrows in an agricultural landscape. The Auk 126:310–318.

Fisher, R. A. (1930). The Genetical Theory of Natural Selection. Clarendon, Oxford, England.

Freeman-Gallant, C. R., N. T. Wheelwright, K. E. Meicklejohn, S. L. States, and S. V. Sollecito (2005). Little effect of extra-pair paternity on the opportunity for sexual selection in Savannah Sparrows (*Passerculus sandwichensis*). Evolution 59:432–440.

Gavin, T., and E. K. Bollinger (1985). Multiple paternity in a territorial passerine: The Bobolink. The Auk 102:550–555.

Graham, E. B., S. P. Caro, and K. W. Sockman (2011). Change in offspring sex ratio over a very short season in Lincoln's Sparrows: The potential role of bill development. Journal of Field Ornithology 82:44–51.

Han, J. I., J. H. Kim, S. Kim, S. R. Park, and K. J. Na (2009). A simple and improved DNA test for avian sex determination. The Auk 126:779–783.

Hanotte, O., C. Zanon, and A. Pugh (1994). Isolation and characterization of microsatellite loci in a passerine bird—The Reed Bunting *Emberiza schoeniclus*. Molecular Ecology 3:529–530.

Hartley, I. R., S. C. Griffith, K. Wilson, M. Shepherd, and T. Burke (1999). Nestling sex ratios in polygynously breeding Corn Bunting *Milaria calandra*. Journal of Avian Biology 30:7–14.

Hasselquist, D., and B. Kempenaers (2002). Parental care and adaptive brood sex ratio manipulation in birds. Philosophical Transactions of the Royal Society B 357:363–372.

Herre, E. A. (1985). Sex ratio adjustment in fig wasps. Science 228:896–898.

Herre, E. A. (1987). Optimality, plasticity and selective regime in fig wasp sex ratios. Nature 329:627–629.

Husby, A., B. E. Sæther, H. Jensen, and T. H. Ringsby (2006). Causes and consequences of adaptive seasonal sex ratio variation in house sparrows. Journal of Animal Ecology 75:1128–1139.

James, W. H. (1975). The distribution of the combinations of the sexes in mammalian litters. Genetics Research 26:45–53.

Jeffery, K. J., L. F. Keller, and P. Arcese (2001). The development of microsatellite loci in the Song Sparrow, *Melospiza melodia* (Aves) and genotyping errors associated with good quality DNA. Molecular Ecology Notes 1:11–13.

Kempenaers, B. (1993). The use of a breeding synchrony index. Ornis Scandinavica 24:1.

Kingma, S. A., M. L. Hall, and A. Peters (2011). No evidence for offspring sex-ratio adjustment to social or environmental conditions in cooperatively breeding Purple-crowned Fairy-Wrens. Behavioral Ecology and Sociobiology 65:1203–1213.

Krackow, S., E. Meelis, and I. C. W. Hardy (2002). Analysis of sex ratio variances and sequences of sex allocation. In Sex ratios. Concepts and Research Methods (I. C. W. Hardy, Editor). Cambridge University Press, Cambridge, UK. pp. 112–131.

Martin, S. G., and T. A. Gavin (1995). Bobolink (*Dolichonyx oryzivorus*). In The Birds of North America, No. 176 (A. Poole and F. Gill, Editors). Academy of Natural Sciences, Philadelphia, and American Ornithologists' Union, Washington, D.C.

Naef-Daenzer, B., F. Widmer, and M. Nuber (2001). Differential post-fledgling survival of Great and Coal Tits in relation to their condition and fledging date. Animal Ecology 70:730–738.

National Agricultural Statistics Service (NASS) (2010). The Census of Agriculture. National Agricultural Statistics Service, US Department of Agriculture. http://www.agcensus.usda.gov/

Neto, J. M., B. Hansson, and D. Hasselquist (2011). Sex allocation in Savi's Warblers *Locustella luscinioides*: Multiple factors affect seasonal trends in brood sex ratios. Behavioral Ecology and Sociobiology 65:297–304.

Neuhäuser, M. (2004). Tests for a biased sex ratio when the data are clustered. Environmental and Ecological Statistics 11:295–304.

Øigarden, T., and J. T. Lifjeld (2013). Primary sex ratios vary with clutch size in the size-dimorphic White-throated Dipper *Cinclus cinclus*. Journal of Ornithology 154:91–97.

Perkins, D. G., N. G. Perlut, and A. M. Strong (2013). Lack of fitness benefits for edge avoidance in nesting grassland birds in the northeastern United States. The Auk 130:512–519.

Perlut, N. G., C. R. Freeman-Gallant, A. M. Strong, T. M. Donovan, C. W. Kilpatrick, and N. Zalik (2008b). Agricultural management affects evolutionary processes in a migratory songbird. Molecular Ecology 17:1248–1255.

Perlut, N. G., L. Kelly, N. J. Zalik, and A. M. Strong (2012). Male Savannah Sparrows reduce parental care in response to paternity loss. Northeastern Naturalist 19:335–344.

Perlut, N. G., A. M. Strong, T. M. Donovan, and N. J. Buckley (2006). Grassland songbirds in a dynamic management landscape: Behavioral responses and management strategies. Ecological Applications 16:2235–2247.

Perlut, N. G., A. M. Strong, T. M. Donovan, and N. J. Buckley (2008a). Grassland songbird survival and recruitment in agricultural landscapes: Implications for source-sink demography. Ecology 89:1941–1952.

Postma, E., F. Heinrich, U. Koller, R. J. Sardell, J. M. Reid, P. Arcese, and L. F. Keller (2011). Disentangling the effect of genes, the environment and chance on sex ratio variation in a wild bird population. Proceedings of the Royal Society of London B 278:2996–3002.

Potti, J., J. A. Dávila, J. L. Tella, Ó. Frías, and S. Villar (2002). Gender and viability selection on morphology in fledgling pied flycatchers. Molecular Ecology 11:1317–1326.

Pyle, P. (1997). Identification Guide to North American Birds, Part I. Slate Creek Press, Bolinas, CA.

Quinn, G. P., and M. J. Keough (2002). Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge, UK.

R Development Core Team (2010). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rutkowska, J., and A. V. Badyaev (2008). Review. Meiotic drive and sex determination: Molecular and cytological mechanisms of sex ratio adjustment in birds. Philosophical Transactions of the Royal Society B 1497:1675–1686.

Saino, N., R. Martinelli, and M. Romano (2008). Ecological and phenological covariates of offspring sex ratio in barn swallows. Evolutionary Ecology 22:659–674.

Shustack, D. P. (2004). Bobolink and Savannah Sparrow habitat selection in the Champlain Valley. M.S. thesis, University of Vermont, USA.

Stauss, M., G. Segelbacher, J. Tomiuk, and L. Bachmann (2005). Sex ratio of *Parus major* and *P. caeruleus* broods depends on parental condition and habitat quality. Oikos 109:367–373.

Taff C. C., C. R. Freeman-Gallant, P. O. Dunn, and L. A. Whittingham (2011). Relationship between brood sex ratio and male ornaments depends on male age in a warbler. Animal Behaviour 81:619–625.

Trivers, R. L., and D. E. Willard (1973). Natural selection of parental ability to vary the sex of offspring. Science 179:90–92.

Wheelwright, N. T., and R. A. Mauck (1998). Philopatry, natal dispersal, and inbreeding avoidance in an island population of Savannah Sparrows. Ecology 79:755–767.

Wheelwright, N. T., and J. D. Rising (2008). Savannah Sparrow (*Passerculus sandwichensis*). In The Birds of North America, No. 45 (A. Poole and F. Gill, Editors). Academy of Natural Sciences, Philadelphia; and American Ornithologists' Union, Washington, D.C.

Wheelwright, N. T., and R. E. Seabury (2003). Fifty:fifty offspring sex ratios in Savannah Sparrows (*Passerculus sandwichensis*). The Auk 120:171–179.

Wilson, K., and I. C. W. Hardy (2002). Statistical analysis of sex ratios: An introduction. In Sex Ratios. Concepts and Research Methods (I. C. W. Hardy, Editor). Cambridge University Press, Cambridge, UK. pp. 49–92.

Yamaguchi, N., K. K. Kawano, K. Eguchi, and T. Yahara (2004). Facultative sex ratio adjustment in response to male tarsus length in the Varied Tit Parus varius. Ibis 146:108–113.

Zalik, N. J., and A. M. Strong (2008). Effects of hay cropping on invertebrate biomass and the breeding ecology of Savannah Sparrows (*Passerculus sandwichensis*). The Auk 125:700–710.