THESIS

SEASONAL VARIATION OF OFFSPRING SEX RATIOS IN EUROPEAN STARLINGS

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY KRISTINA MCKINLEY ENTITLED "SEASONAL VARIATION OF SEX RATIOS IN EUROPEAN STARLINGS" BE ACCEPTED AS FULFILLING PARTIAL REQUIREMENTS FOR THE THESIS IN ZOOLOGY.

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Abstract

I determined offspring sex ratios of a Northern Utah population of European Starlings (*Sturnus vulgaris*) during the 2001 breeding season. Genomic DNA isolation, polymerase chain reaction (PCR), and agarose gel electrophoresis were used to determine the sex of each nestling. A total of 139 nestlings from 34 broods were successfully sexed. Among nests that exhibited no mortality, the sampled population consisted of 46.8% males and 53.2% females, which was not significantly different from parity ($G=0.290$, $p>0.05$). Overall these data provide support for an equal offspring sex ratio. Furthermore, I was unable to find any support for facultative manipulation of offspring sex ratios by individual females.

Introduction

Sex ratio theory has many adaptive hypotheses that have been applied in research studies to explain sex ratios in avian populations. In 1930, R.A. Fisher proposed the first adaptive explanation for the widespread occurrence of similar numbers of male and female offspring in natural populations. This hypothesis proposes that parents will allocate resources equally between both male and female offspring. When the sexes are equally costly to raise, parents should produce equal numbers of each; however, when the costs differ they should produce more of the cheaper sex (Fisher, 1930). A study on sex ratios in Lesser Black-backed Gulls (*Larus fuscus*) revealed that female chicks were smaller, and therefore cheaper, than male chicks to raise. The sex ratio was female-biased at the time of fledging in this population, which suggests that more of the cheaper sex is being invested in. This example shows support for Fisher's hypothesis, although

the investigators treat the results with caution as the study may have been affected by such factors as food variability and weather (Griffiths, 1992).

Trivers and Willard (1973) extended the work of Fisher (1930) and hypothesized that natural selection should favor the ability of parents to modify the sex ratio of their offspring. This hypothesis provided an adaptive explanation for unequal sex ratios within a brood rather than at the population level. They proposed that parents in good condition will invest more heavily in the sex with the highest reproductive variance (variation in offspring produced). A female in good condition should be able to produce higher quality offspring than a female in poor condition. There are three assumptions of the Trivers and Willard hypothesis. The first is that the condition of the young after parental investment correlates to the mother's condition. Second, the young's condition at the end of parental investment will endure to adulthood. Third, the sex with the highest reproductive variance in good condition is helped more in reproductive success relative to the sex with lowest reproductive variance (Trivers and Willard, 1973). Many studies that have confirmed the Trivers and Willard hypothesis involve sexually dimorphic species. A study conducted by Korpimaki et al. (2000) supports this hypothesis. Parents of Eurasian Kestrels (*Falco tinnunculus*) adjusted offspring sex ratio toward an excess of the cheaper sex when food availability was low. Furthermore, parents in good condition tended to produce an excess of female offspring (females are larger and thus are more costly to raise) and therefore there is a correlation between parental body condition and sex ratio.

Studies have demonstrated that both primary (before hatching) and secondary (after hatching) sex ratio adjustment occurs in birds. Krackow (1995) proposed some

potential mechanisms of primary sex ratio manipulation. In birds females are the heterogametic sex (ZW) and males are homogametic (ZZ). He proposed that females may be able to shed Z- and W-bearing ova non-randomly to control ovulation sequence or that tubal synchrony (ova at the beginning and ending of the ovulation sequence may be at a higher risk for developmental failure) may be a factor. Additionally, birds may also modify the secondary sex ratio by preferentially feeding one of the sexes. Support for adjusting the secondary sex ratio was found in Zebra Finches (*Poephila guttata*) whose parents preferentially fed offspring depending on the quality of the male (Burley, 1986).

This study tested both the Fisher hypothesis at the population level and the Trivers and Willard hypothesis at the level of the individual brood on a population of European Starlings (*Sturnus vulgaris*). Because I am testing at the level of the individual brood and at the population level these two hypotheses are not mutually exclusive.

Until recently it was impossible to accurately determine the sex of most avian species before fledging. European Starlings are distinguishable as adults in the breeding season but not as nestlings. Because of this limitation, genomic DNA isolation, polymerase chain reaction (PCR), and agarose gel electrophoresis were used to sex the nestlings and test predictions of both hypotheses. The *CHD* gene is a sex-linked gene that encodes for a chomo-helicase-DNA (CHD) binding protein. It is designated as *CHD1W* on the W chromosome and *CHD1Z* on the Z chromosome. The gene appears on both the Z and W chromosomes in avian females and on the Z chromosomes in avian males. Due to the presence of a length polymorphism in one of the introns, *CHD1W* is

smaller than CHD1Z. This difference is apparent when using PCR primers that flank the polymorphic intron (Fridolfsson and Ellegren, 1999).

Because European Starlings are monomorphic in body size, the Fisher hypothesis predicts that the sex ratio of offspring at the population level will not be different from parity. This will indicate that the cost of raising each sex is equal.

If parents can facultatively manipulate the sex ratio of their offspring, then the Trivers and Willard hypothesis predicts that females in good condition should skew the sex ratio of their individual brood toward the sex that will have the highest reproductive success. Furthermore, parents in good condition should invest in the sex with the highest reproductive variance. Male European Starlings are generally thought to have the greatest reproductive variance because some male members of the population do not reproduce whereas other males are polygynous. Most females Starlings breed every year (Cavitt personal communication). Therefore, in this population a female in good condition will benefit most from investing in male offspring while a female in poor condition will benefit most from investing in female offspring.

Materials and Methods

Study site and species

This study was conducted during the 2001 breeding season on a population of European Starlings at the Abbey of Our Lady of the Holy Trinity Monastery in Huntsville, Utah (41° 14'N, 111° 42'W). Ninety-eight rectangular nest boxes (36x18x14 cm) with removable lids and a sliding metal door used to trap adults inside were installed on fence rows and on out-buildings.

European Starlings are an ideal subject for study because they are a very abundant and widespread species, they tolerate human disturbance at the nest, and because they are secondary cavity-nesting birds they readily accept nest boxes. Egg laying is synchronized within this population and first clutches are initiated in late April (early season) and second clutches are initiated in mid-June (late season) (Cavitt personal communication). Clutch size ranges from 2-8 eggs. Incubation begins with the penultimate egg and continues for 12 days. Nestlings are fed by one or both parents until nest leaving. Young leave the nest after 16-25 days but continue to be fed by one or both parents for an additional one to two weeks (Cabe, 1993).

General Field Procedures

Nest boxes were checked every three to four days from early March to late July. At these checks information was recorded concerning the status of existing nests and the initiation of new nests. Clutch size was assigned when the same number of eggs was present on consecutive visits and there was evidence that incubation had commenced (i.e. parent behavior and egg temperature).

Nestlings were weighed and banded with a numbered US Fish and Wildlife service leg band at twelve days. In addition, culmen, tarsus, and wing length measurements were recorded to the nearest millimeter. Also at this age, 30 microliter blood samples were obtained for molecular sex determination from the nestling's brachial vein. This blood sample was stored in a buffered solution (RGB buffer, 50mM EDTA, 2% SDS, 50mM Tris pH 8) at -20°C. Females from each nest were captured during late incubation and were banded, weighed, and wing length measurements were recorded. A condition index was calculated by dividing female mass by female wing length. Females

were also classified according to breeding experience. Females that were recaptured and known to have bred on the study site during a previous year were considered experienced while unbanded females were considered inexperienced.

Molecular Sex Determination

For DNA isolation 10μL of 10mg/mL proteinase K, 30μL 10% SDS, and 500μL SET (100mM NaCl, 1mM EDTA, 100mM Tris-HCl pH 8) was added to the buffered blood mixture and was incubated at 55°C in a water bath for 20 minutes. Following this incubation the supernatant was extracted twice with an equal amount of phenol and chloroform and twice with only chloroform. Nucleic acids were precipitated by adding 1000μL 100% ethanol, centrifuging for 2 minutes, and decanting the supernatant. The pellet was washed with 500μL of 70% ethanol, centrifuged for 5 minutes, and the ethanol decanted. After drying, the pellet was resuspended in 100μL of TE buffer (1mM EDTA, 10mM Tris-HCl pH 8).

The isolated DNA was used in PCR amplification of the *CHD* gene. Two PCR primers, specific for the *CHD* locus, were obtained from SIGMA/GENOSYS. These two primers are 2550 F (5'-GTTACTGATTCGTCTACGAGA-3') and 2718 R (5'-ATTGAAATGATCCAGTGCTTG-3') and were prepared at a working concentration of 320ng/μL. All amplifications were carried out in 25μL volumes in a Perkin Elmer Thermal Cycler using the following cycling profile: 94° for 1 minute, 50° for 1 minute, and 74° for 1 minute. This temperature profile was repeated for 30 cycles.

Agarose gel electrophoresis is used to visualize the amplified DNA. The concentration of the gel used in this study was a 1.5% gel (30 mL TAE buffer and 0.45 g agarose). Following electrophoresis, DNA fragments were visualized by ethidium

bromide staining. Females are identified on the gel as having one band at 450 base pairs and another at 650 base pairs. Male nestlings are identified on the gel as having only one band at 650 base pairs (Fig. 1).

Statistical Methods

G-tests were used to test if sex ratios deviated from parity in the early, late, and early and late seasons combined. I used regression analysis and ANOVA to test for sex ratio differences in relation to date of nest initiation, female mass, female condition, and female experience. An analysis of variance was used to examine if sex ratios differed between nests that experienced partial brood losses and those with no nestling mortality. All brood sex ratios are expressed as the proportion of males in a nest.

Results

The sex was successfully determined for 139 nestlings from 34 nests. The sex ratio of offspring did not significantly deviate from parity during either the early ($G=0.143$, $p>0.05$) or late seasons ($G=0.152$, $p>0.05$). These results are shown graphically in Figure 2. Furthermore, the sex ratio of offspring did not deviate from parity when both seasons are combined (46.8% male, 53.2% female, $G=0.290$, $p>0.05$).

In the early season, there were no significant relationships between proportion of male offspring and Julian day of nest initiation ($F_{1,20}=0.258$, $p=0.617$), female mass ($F_{1,16}=0.449$, $p=0.512$), or female condition ($F_{1,16}=0.122$, $p=0.731$). There were no significant differences found in the late season between proportion of male offspring and Julian day of nest initiation ($F_{1,10}=0.010$, $p=0.923$), female mass ($F_{1,7}=0.079$, $p=0.786$), or female condition ($F_{1,6}=0.017$, $p=0.901$). In the overall population there were no

significant differences seen between sex ratio and Julian day of nest initiation (Fig. 3, $F_{1,32}$=0.072, p=0.790), female mass ($F_{1,25}$=0.510, p=0.482), or female condition (Fig. 4, $F_{1,24}$=0.212, p=0.649).

To determine if one sex is more vulnerable to mortality, sex ratios were examined in nests exhibiting partial nestling mortality and in nests with no mortality. For nests with partial mortality I found no deviation from parity during the early ($F_{1,20}$=0.186, p=0.671), late ($F=_{1,10}$=0.004, p=0.952), or with early and late seasons pooled ($F_{1,19}$=2.386, p=0.139).

Analyses comparing relationships between sex ratio and experience of female showed no significant differences in the early season ($F_{1,11}$=0.000, p=0.996) or in the late season ($F_{1,6}$=0.178, p=0.687).

Tests were performed to determine whether brood mass, number of young fledged, or brood size had an affect on sex ratio. There were no significant differences between sex ratio in nests that were female-biased, male-biased, or of equal sex ratio in relation to brood mass ($F_{2,18}$=1.128, p=0.345), number of young fledged ($F_{2,18}$=0.053, p=0.948), or brood size ($F_{2,18}$=1.067, p=0.365). Additionally, no significant relationships were found between sex ratio and brood mass ($F_{1,19}$=0.512, p=0.483), number of young fledged ($F_{1,19}$=0.001, p=0.980), or brood size ($F_{1,19}$=0.444, p=0.513).

Discussion

The overall proportion of male and females nestlings in this population did not deviate from parity, which shows support for Fisher's suggestion of equal sex ratios (Fisher, 1930). A study conducted on Savannah Sparrows (*Passerculus sandwichensis*)

show similar findings over a 14 year period (Wheelwright and Seabury, 2003). Furthermore, Wheelwright and Seabury (2003) were unable to find that timing of breeding, parental age, or mating status affected sex ratio in this species as predicted by Trivers and Willard (1973).

In this population no relationship was found between sex ratio and timing of breeding. This data correspond to a similar study on House Sparrows (*Passer domesticus*) where there were no correlations found between sex ratio and date of first egg (Westneat et al., 2002). Several other studies have shown that as the season progresses and food availability becomes limited, the sex ratio becomes biased toward the cheaper sex. Sex ratios of Eurasian Kestrel broods (females are larger than males in this species) were found to be male-biased in years where food availability was unfavorable (Korpimaki et al., 2000). Additionally, female Blue-footed Booby (*Sula nebouxii*) chicks have been found to suffer greater mortality than males because their larger size renders them more costly to raise (Torres and Drummond, 1997).

I found no support for the Trivers and Willard hypothesis. There were no significant relationships between sex ratio and female mass, condition, or breeding experience. Leech et al. (2001) reached similar conclusions in their study on the Blue Tit (*Parus caeruleus*). They found no significant effects on brood sex ratio when compared to parental quality or brood size. These findings are contrary to findings of Korpimaki et al. (2000) in which it was found that Kestrel parents in good condition had female-biased sex ratios because females were larger in size and benefited more by being in good condition. Blue Tits and European Starlings are not sexually dimorphic in body size, thus it may be that neither sex benefits more from being in good condition in these species.

In conclusion, the overall offspring sex ratio of this population of European Starlings was not significantly different from 50:50. These findings are consistent with Fisher's notion of equal sex ratio at the population level (Fisher, 1930). However, we were unable to find any support for the Trivers and Willard hypothesis. It is important to report results that do not show support for adaptive modification sex ratio models to further the understanding of sex ratio manipulation in avian populations.

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Wheelwright, N.T. and R.E. Seabury. 2003. Fifty:fifty offspring sex ratios in Savannah Sparrows (*Passerculus sandwichensis*). The Auk 120(1):171-179.

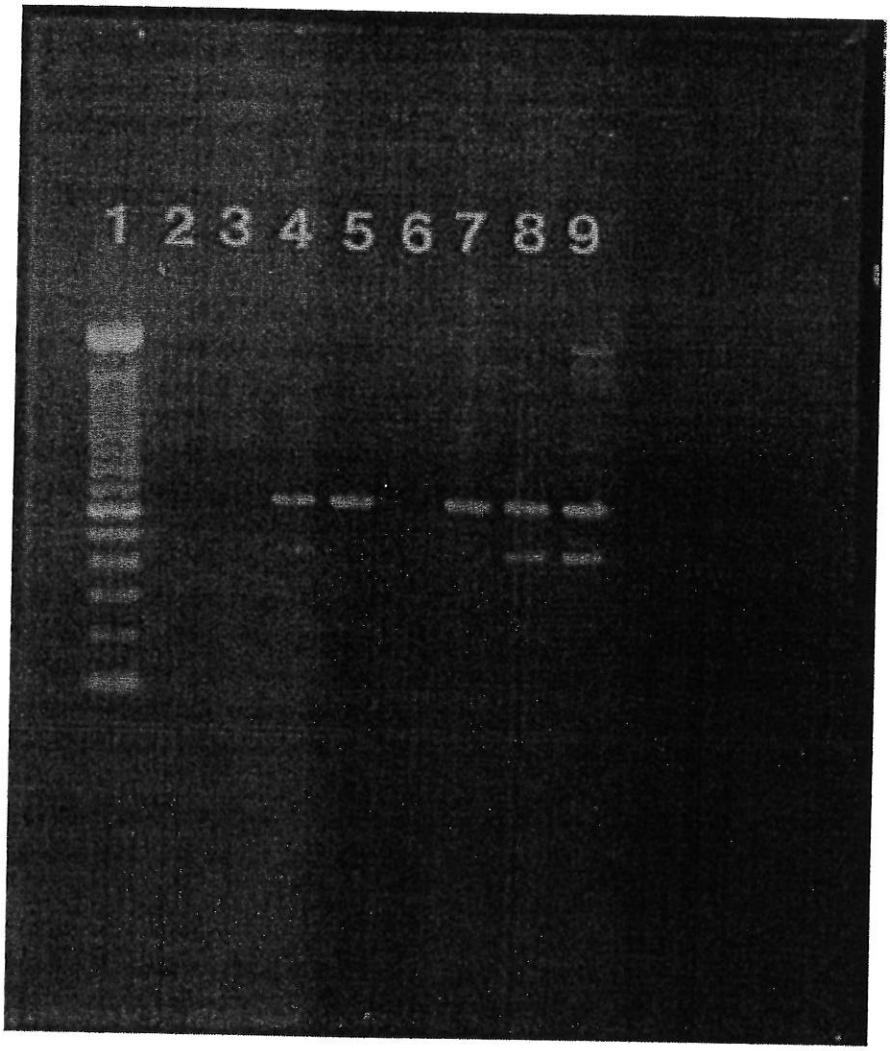

Figure 1. An agarose gel representing five samples of amplified DNA. Lane 1 contains a 100 base-pair ladder. Lanes 4,8, and 9 are female nestlings and lanes 5 and 7 are male nestlings.

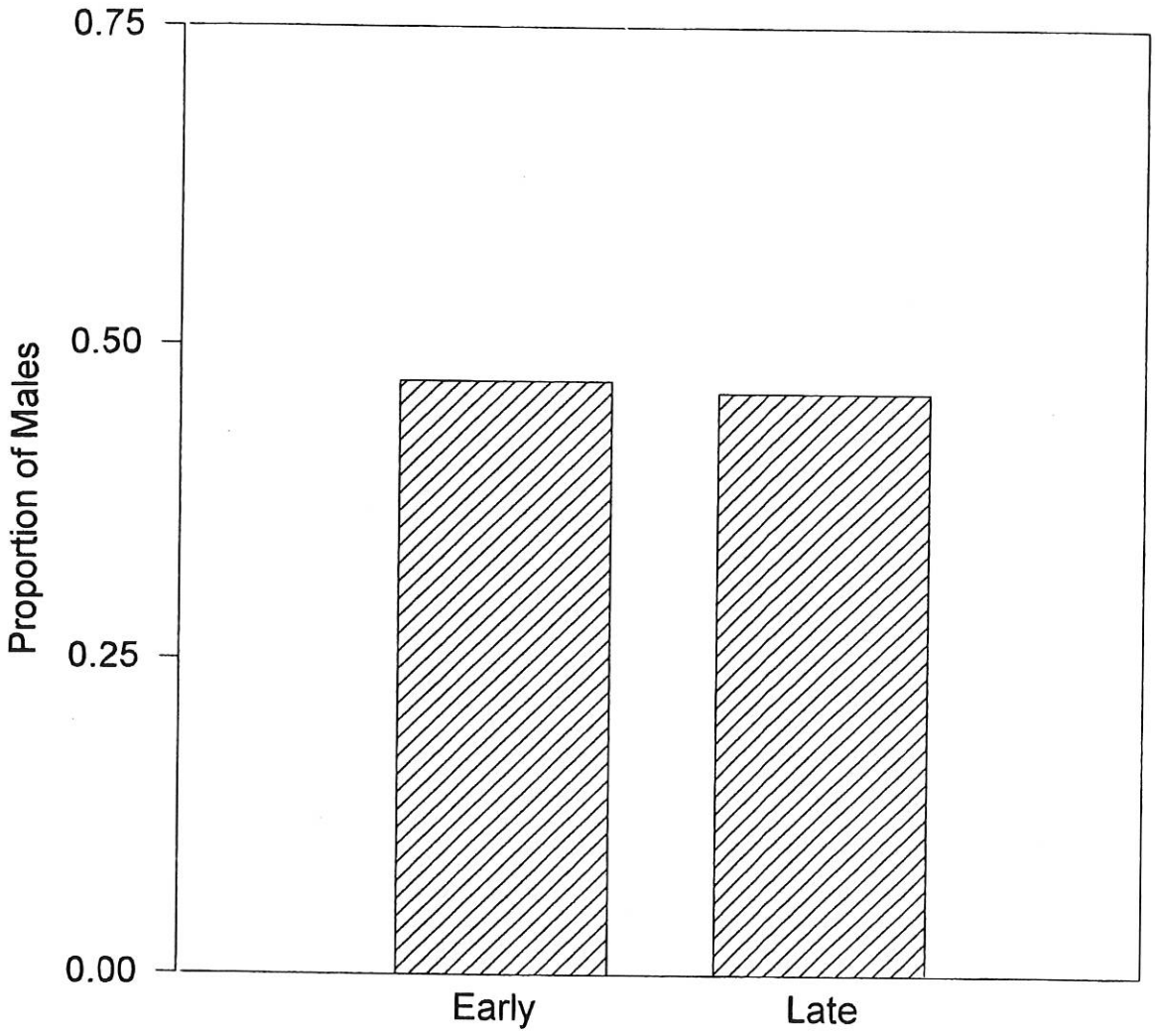

Figure 2. Proportion of male nestlings in the early and late seasons ($p>0.05$).

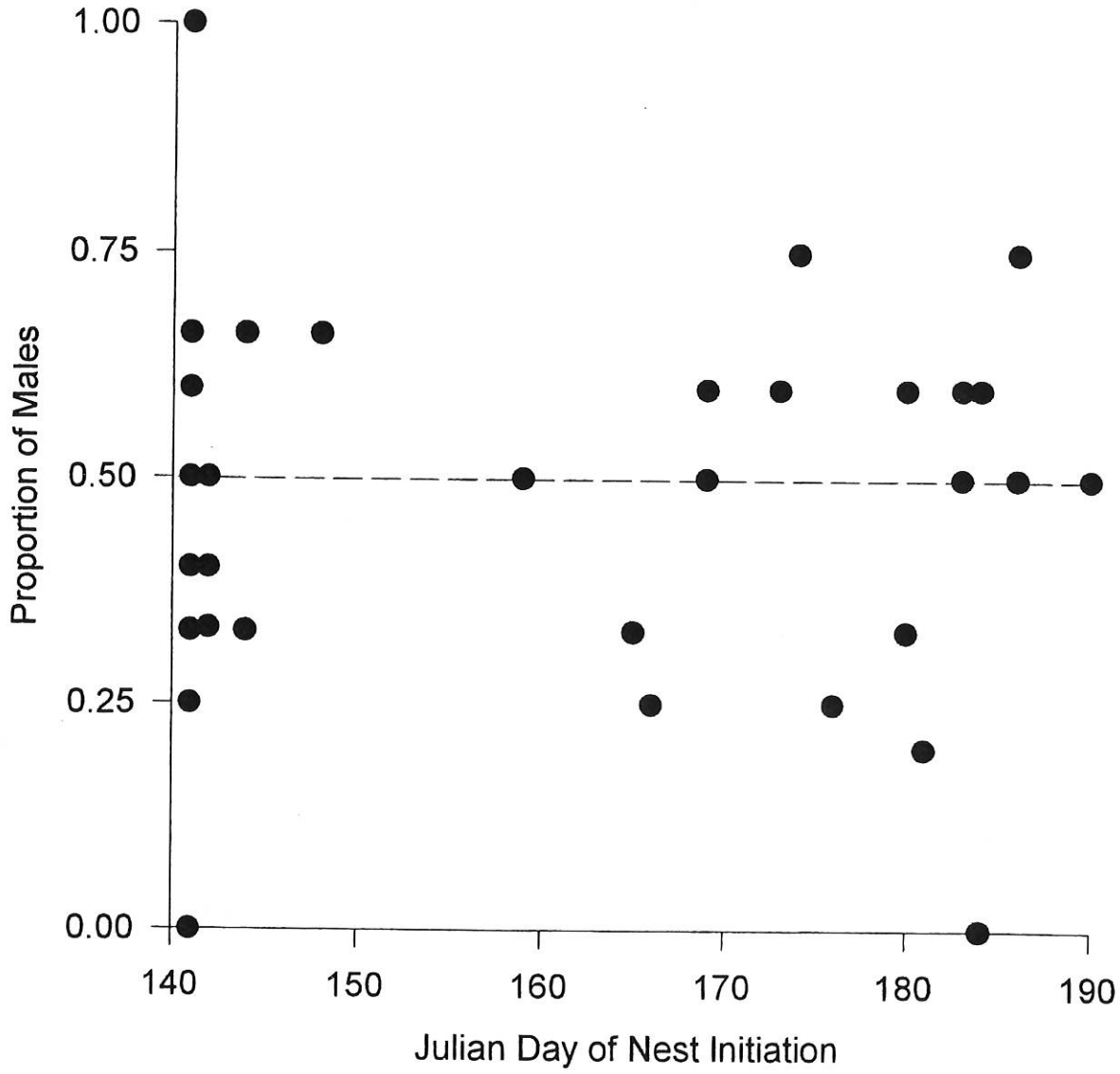

Figure 3. Relationship between sex ratio and Julian Day of Nest Initiation. Each point represents the proportion of males in an individual nest. Nests that lie above the dashed line are male-biased and nests that lie below the line are female-biased ($p>0.05$).

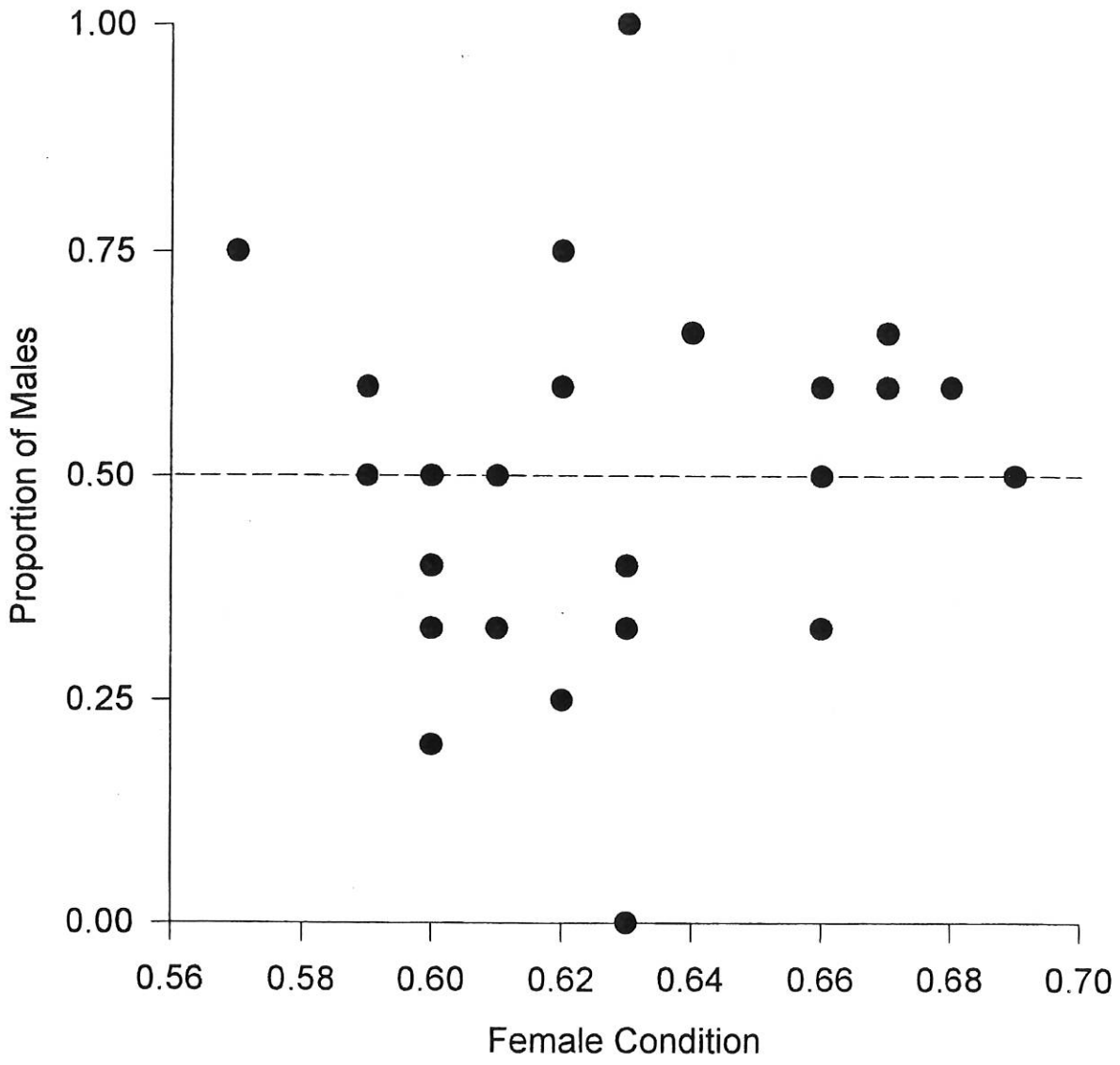

Figure 4. Relationship between sex ratio and female condition (mass/wing). Each point represents the proportion of males in an individual nest. Nests that lie above the dashed line are male-biased and nests that lie below the line are female-biased ($p>0.05$).