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PARENTAL INVESTMENT AND OFFSPRING SEX RATIOS IN HOUSE
SPARROWS, *PASSER DOMESTICUS* AND CATTLE EGRETS, *BUBULCUS IBIS*

A Dissertation

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By

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PARENTAL INVESTMENT AND OFFSPRING SEX RATIOS IN HOUSE
SPARROWS, *PASSER DOMESTICUS* AND CATTLE EGRETS, *BUBULCUS IBIS*

A Dissertation APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

BY

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PREFACE

The five chapters of this dissertation have been prepared in submission format and
include co-authors. Chapters I and V are being submitted to the Auk. Chapter II is
being submitted to Behavioral Ecology and Sociobiology, chapter III is being submitted
to Animal Behavior and chapter IV is being submitted to Behavioral Ecology.

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Abstract

The problem of natural selection and sex ratios was raised by Darwin in his 1871 book entitled The Descent of Man, and he concluded "...I see now that the whole problem is so intricate that it is safer to leave its solution for the future." Fisher (1930) was the first to take this challenge and to try to make sense of the fact that parents tend to produce male and female offspring in nearly equal numbers. His argument that equal investment in the two sexes is maintained through frequency-dependent selection laid the ground work for all future research on the theory of sex allocation. Since Fisher, others have proposed conditions under which parents might be selected to bias their investment in the two sexes. I examined brood sex ratios and parental investment patterns in two species of birds: house sparrows, *Passer domesticus* and cattle egrets, *Bubulcus ibis*.

I found that, at the population level, house sparrows do not vary brood sex ratios over the course of the breeding season but there is heterogeneity among females, indicating that individual females are producing broods with biased sex ratios. If sex ratios are not biased in relation to time in the breeding season, are there other factors correlated to brood sex ratios? It has been suggested that females might bias the sex ratio of offspring in relation to the attractiveness of their mates: females mated to more attractive males should bias the sex ratio in favor of sons, whereas females with less attractive mates should produce more daughters. In house sparrows, females reportedly prefer to mate with males with larger black throat badges. I tested whether females bias the sex ratio of their brood in relation to the badge size of their mate. Females mated to larger-badged males did produce more sons and females mated to smaller-badged males tended to have more daughters. So females are biasing the sex ratio of offspring in relation to male badge size, but are they gaining any other benefits from being mated to large-badged males? I tested whether female house sparrows gain direct benefits from choosing large-badged males by observing male parental behavior.

Larger-badged males contributed proportionately more to the feeding of nestlings and fledged more of the young that hatched in their nest than smaller-badged males. This suggests that females may gain direct benefits from choosing large-badged males.

While I have shown that house sparrows can produce biased brood sex ratios, sex allocation also involves the investment in raising offspring to independence. I looked for differences in parental investment in sons and daughters during the nestling period by manipulating brood sex ratios and observing parental feeding rates and nestling growth rates. There were no differences in maternal and paternal feeding rates to all-male, all-female, and mixed-sex control nests at any time during the nestling period. Nor were there differences in final weights of nestlings or in nestling growth rates over the course of the experiment. I concluded that there was not biased allocation during the period of parental care. In house sparrows, differential allocation may be expressed in biased brood sex ratios rather than in differential parental investment during the nestling period.

Lastly, I looked at sex ratio variation in relation to hatch order in a species with asynchronous hatching, cattle egrets. Cattle egrets exhibit facultative siblicide and the first-hatched (a-) and second-hatched (b-) chicks receive more food and are more likely to survive the nestling period than the last-hatched (c-) chick in three chick broods. I found that females bias the sex ratio of the a-chick in favor of sons and that overall sex ratios are male-biased due to this bias in the sex of the a-chick and the lack of a corresponding bias towards daughters in the other ranks. This result is consistent with one possible mechanism for biasing the sex ratio (resorption or abandonment of first laid eggs of the less advantageous sex), although the question of how house sparrow females systematically bias their brood sex ratios remains unanswered.

Do house sparrows, *Passer domesticus*, vary brood sex ratio during the breeding season?

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Abstract. Seasonal variation in sex ratios has been observed in several bird species. Temporal variation in food availability combined with differential costs of raising the two sexes may influence variation in brood sex ratio over the breeding season. Dispersal differences between male and female fledglings and the resulting local resource competition with parents may also favor seasonal variation in sex ratios. Using flow cytometry to determine hatchling gender, I examined brood sex ratios at hatching in relation to time during the breeding season for 112 broods of house sparrows, *Passer domesticus*. There was no significant effect of hatching dates on the proportion of males in a brood nor was there any difference in sex ratios among early-, mid-, and late-season nests. Overall sex ratios did not differ from 0.5 for any year in the study but there was some evidence of heterogeneity among broods, indicating that some individual females may be producing broods with biased sex ratios, irrespective of the time in season.

Introduction

Fisher (1958) predicted equal investment by parents in male and female offspring. If there are differences in the cost of raising the two sexes, sex ratios biased in favor of the less expensive sex are expected. Sex ratio variation in birds has been examined in response to sexual size dimorphism in nestlings (Dijkstra et al., 1990; Olsen and Cockburn, 1991; Zijlstra et al., 1992; Wiebe and Bortolotti, 1992; Rosenfield et al., 1997), differential dispersal of male and female fledglings (Gowaty, 1993), maternal age and breeding experience (Blank and Nolan, 1983; Komdeur, 1996), and mate attractiveness (Burley, 1986; Ellegren et al., 1996). Environmental conditions (e.g. food availability) may vary within a single breeding season and thus influence the cost of raising offspring. Environmental conditions combined with other factors that have been linked to sex ratio variation, such as sexual size dimorphism, may influence the optimal sex ratio for parents, especially in taxa with extended breeding seasons. Seasonal variations in brood sex ratios have been reported for common grackles, *Quiscalus quiscula* (Howe, 1977), red-winged blackbirds, *Agelaius phoeniceus* (Weatherhead, 1983), great tits, *Parus major* (Lessells et al. 1996), black-billed magpies, *Pica pica* (Gerstell and Trost, 1997) and several raptors, including peregrine falcons, *Falco peregrinus* (Olsen and Cockburn, 1991), European kestrels, *F. tinnunculus* (Dijkstra et al. 1990), American kestrels, *F. sparverius* (Wiebe and Bortolotti, 1992) and marsh harriers, *Circus aeruginosus* (Zijlstra et al. 1992). In many studies of sex ratio variation it is difficult to determine the cause of the bias and to distinguish between differential mortality of the two sexes during the nestling period and parental manipulation of the sex ratio at laying. Recent advances in molecular techniques have allowed the identification of the sex of nestlings before substantial mortality occurs (i.e. Lessells et al. 1996) and thus give strong evidence for parental manipulation of the sex ratio at laying.

Size dimorphism between male and female nestlings may result in sex ratios biased in favor of the smaller sex when food is restricted and sex ratios biased in favor of the larger sex when food is abundant (Howe 1977; Olsen and Cockburn 1991). Different dispersal patterns may also affect sex ratio variation if one sex disperses farther or at an earlier time in the season than the other sex (Gowaty, 1993). Parents may bias the sex ratio in favor of the dispersive sex at a time in the season when they are more likely to disperse successfully. In these situations, parents also decrease the likelihood of local resource competition with offspring if they bias the sex ratio in favor of the dispersing sex. In taxa where male and female offspring differ in their probability of breeding as a yearling, seasonal sex ratio variation has been attributed to the influence hatch date might have on that probability for sons versus daughters (Daan et al. 1996; Dijkstra et al. 1990; Zijlstra et al. 1992). Finally, maternal age can affect sex ratios (Blank and Nolan 1983; Cooch et al. 1997) and if age affects when females reproduce during the breeding season, that factor might also influence seasonal sex ratios.

House sparrows initiate egg-laying in Oklahoma in late March and continue breeding through July. The environmental conditions change during this four-month breeding season from cool and wet in March and April to extremely hot and dry in mid-summer and the optimal sex ratio may vary with those conditions. House sparrows also form winter feeding flocks with dominance hierarchies in which subadult birds are subordinate to older birds and males are dominant to females (Lowther and Cink 1992). The amount of time that fledglings have before dispersal generally occurs and juvenile feeding flocks form depends on when they hatch during the breeding season. If this affects their body size and condition, it might also affect their position in the dominance hierarchy and their access to food sources in the fall and winter months. I used flow cytometry to examine brood sex ratios in relation to hatch date for house sparrows breeding in central Oklahoma. I hypothesized that the proportion of females in a brood

would decrease as the breeding season progressed. In some house sparrow populations, females disperse farther than males (Lowther 1979) and if early-born females are more likely to disperse, local resource competition with parents would be reduced if more females are produced in early broods. Early born females might also have more time to build up reserves for dispersal or to survive the winter in feeding flocks in which they are the subordinate members.

Methods

This study was conducted from the summer of 1994 - 1997, using box-nesting populations of house sparrows (*Passer domesticus*) in Norman, OK. For each sampled brood, I clipped a toenail of each nestling to collect a small blood sample (< 50 µl) in ACD anticoagulant. Samples were collected from all nestlings when they were 1-3 days old and stored at 4° C until analysis. I collected blood from some broods when the nestlings were older (9-10 days old) but only if no brood reduction had occurred. The sex of each nestling in the study was identified using flow cytometry, following the protocol established in Tiersch et al. (1989). Sparrow blood samples were combined with an internal reference sample of catfish (*Ictalurus punctatus*) blood and stained with 0.5 ml of stain containing 0.1% sodium citrate, 0.1% Triton-X100, 25 µl RNAse and 25 µg propidium iodide. The stained cells were kept at room temperature and analyzed within 30 minutes.

The average DNA content per cell for each sample was calculated using the median channel of the fluorescence intensity peaks according to the formula: pg sparrow DNA = (B/C)*2.0 pg, where B=bird median channel, C=catfish median channel and 2.0 pg is the DNA content of catfish (Tiersch et al. 1989). DNA content for each unknown house sparrow nestling was compared to known-sex samples run concurrently. These known-sex samples were from adults in the population that had

been ground trapped and bled that same year. If there was some ambiguity in assigning sex to a given sample, the nestling's score was compared directly to that of its parents (if available) or its siblings. If sex of the nestling was still undetermined after these steps, neither the chick nor the brood it came from were used in the analyses. Once sex was identified for all nestlings, brood sex ratios were calculated as the proportion of the brood that was male.

Hatch day was measured as the number of days after the first egg hatched in each season. This date was 8 April in 1994, 7 April in both 1995 and 1996, and 12 April in 1997. Broods were also grouped as early-season (hatched 1 to 30 days after the season started), mid-season (31-60 days) and late-season (hatched after day 61). Brood sex ratios were determined for 112 house sparrow broods: 9 in 1994, 43 in 1995, 34 in 1996, and 26 in 1997. I only sampled one brood per nestbox per year, so although the data set includes broods from throughout the season, and presumably first, second, and third broods, they are from different females. Sex ratios were analyzed using a G-test with William's correction (Sokal and Rohlf 1981) and all other analyses were performed using JMP 3.2 (SAS Institute Inc).

Results

The sex ratio (proportion of brood that was male) of chicks hatched in sample nests for each year from 1994-1997 was 0.47, 0.49, 0.45, and 0.48 respectively. None of these deviated significantly from 0.5 (1994: $G_{adj} = 0.116$, p = 0.73; 1995: $G_{adj} = 0.052$, p = 0.81; 1996: $G_{adj} = 1.397$, p = 0.23; 1997: $G_{adj} = 0.147$, p = 0.70). There was no relationship between brood sex ratio and hatch day for any of the individual years in this study (Figure 1) nor for all years combined ($r_s = 0.07$, p = 0.44, n = 112) (Figure 2). Although the p-value for 1994 is low, the data were from only 9 nests and thus the

biological significance of this correlation is questionable. There were no differences among the 4 years of the study in brood sex ratios (Kruskal-Wallis χ^2 = 0.892, p = 0.83) and with all years combined the sex ratio did not deviate from 0.5 (mean sex ratio = 0.47; G_{adj} = 1.167, p = 0.28, n = 112).

There are three main periods of hatching in these populations, corresponding approximately to mid-April, late May, and late June/early July. Accordingly, I separated the 112 nests into early-season (up to 30 days after the first eggs hatched in a year), mid-season (hatch days between 31 and 60), and late-season nests (hatching more than 61 days after the first eggs of the season). There were 12 early-season, 61 mid-season, and 39 late-season nests. There were no significant differences in the brood sex ratios for the three time periods (χ^2 = 1.859, p = 0.39) (Figure 3), nor did the sex ratio differ significantly from parity for any time period (early: G_{adj} = 0.089, p = 0.77; mid: G_{adj} = 2.089, p = 0.14; late: G_{adj} = 0.026, p = 0.87).

There was some evidence of heterogeneity in sex ratio among broods (G_H = 132.85, p = 0.07, n = 112), indicating that although the population (pooled) sex ratio is not different from 0.5, individual females are producing brood sex ratios that are differ from other females in the population. If each brood size is analyzed separately , the only brood size with significant heterogeneity among broods is the three-chick brood (G_H = 42.85, p = 0.01, n = 25). Neither the four-chick (G_H = 64.92, p = 0.22, n = 58) nor the five-chick broods (G_H = 24.34, p = 0.61, n = 28) had significant differences between broods in the sex ratios at hatching.

Discussion

Brood sex ratios did not vary with hatch day and the sex ratios from early-, mid- and late-season nests did not deviate significantly from parity. Because female house sparrows are reported to be more likely to disperse than males (Lowther 1979), I

hypothesized that sex ratios would be female-biased early in the season. There is evidence, though, that this dispersal pattern is not present in this central OK population studied. Of the over 800 nestlings banded between 1994 and 1996, 27 have been resighted near their natal area. These included 17 females and 10 males. Given that the mean sex ratio at hatching did not vary between years nor did it deviate from 0.5, these returns suggest that females are not more likely to disperse than males in this population. If so, then parents may have no reason to bias the sex ratio in response to the time in the breeding season.

In other studies, seasonal sex ratio variation has sometimes been attributed to the influence of hatch date on the age at first breeding, when the probability that an individual will breed as a yearling varies between sons and daughters. In European kestrels, increasing hatch date decreased the chances of breeding in the first year for males but not for females and the proportion of males in a brood declined over the breeding season (Dijkstra et al. 1990). The opposite pattern is seen in marsh harriers, with hatch date influencing first-year female breeding prospects but not male (Zijlstra et al. 1992), and these sex ratios declined as the season progressed. The influence of hatch date on juvenile recruitment into the breeding population is unknown for house sparrows, although these results indicate that even if a difference exists, it does not result in seasonal variation in sex ratios.

Food availability may also limit the amount of food parents are able to deliver to the nest and thus sexual size dimorphism could influence the optimal sex ratio at different times during the breeding season. In some areas, food abundance is unpredictable and it is parental quality or breeding environment that determine the amount of food that is available to offspring. In the green woodhoopoe, *Phoeniculus purpureus*, females are smaller than males and breeding groups with fewer helpers produced broods with a female-biased sex ratio (Ligon and Ligon 1990). In the Seychelles warbler, *Acrocephalus sechellensis*, parents adjust the sex of their offspring

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in response to territory quality and the ability of the territory to support additional helpers-at-the-nest (Komdeur et al. 1997). These helpers are mostly females and parents on high-quality territories produce more daughters than pairs on low quality territories, where the presence of helpers would actually decrease the fitness of the breeding pair. In American kestrels, smaller females and parents in poor condition were more likely to have broods biased in favor of the smaller sex, males (Wiebe and Bortolotti 1992). In this same study, food abundance did not vary predictably with time in season but did correlate with brood sex ratio at hatching. Broods produced during times with low food abundance were male-biased. House sparrow fledglings reportedly have a slight size dimorphism during the nestling period (Fleischer et al. 1984), with males being slightly larger, but experimental work has shown that broods composed of all-male nestlings do not receive more feeding visits from parents than either all-female broods or mixed-sex broods (Voltura, Chapter 4). Again, this may explain why parents do not vary the sex ratio over the breeding season. Food shortages or abundance may affect male and female nestlings equally and thus no seasonal sex ratio bias would be expected.

Although house sparrow brood sex ratios did not vary with time in season, there was some heterogeneity among females indicating individual females do produce biased brood sex ratios. Female house sparrows reportedly prefer to mate with large-badged males (Møller, 1988) and another study on sex ratios showed that females mated to large badged-males produce broods with male-biased sex ratios (Voltura et al., chapter 2). Hatch day does not affect the relationship between male badge size and brood sex ratio. If larger-badged males initiated their first clutch earlier in the season than males with smaller badges, a male-biased sex ratio would be expected early in the season and progressively more females should be produced later. But because house sparrows lay multiple clutches in a single season, it is possible some of these relationships will not be evident from this data set which includes broods from

throughout the breeding season. Similarly, we do not know the ages of most of the individuals breeding in this population and any influence of maternal age or breeding experience on the sex ratio is unknown. In red-winged blackbirds, older females produced male-biased broods and younger, less-experienced breeders produced more daughters (Blank and Nolan 1983). So far the only factor to correlate with brood sex ratios for house sparrows is the badge size of the male at the nest, but the age of the parent and the influence of hatch date on survival of nestlings to breeding age needs to be examined for this population.

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Figure legends

Figure 1.   Sex ratios, measured as the proportion of males in a brood,
                     correlated with hatch day for each of the four years of this study

Figure 2.   The proportion of males in a brood correlated with hatch day for
                     all years combined (n=112).

Figure 3.   Comparison of the sex ratios of early-, mid- and late-season
                     nests (Kruskal-Wallis χ^2 = 1.859, p=0.39, n=112). Error bars
                     are ±2 SE.
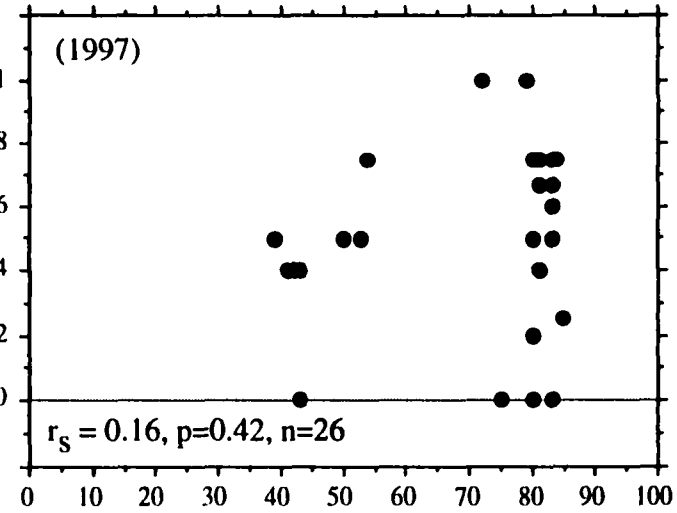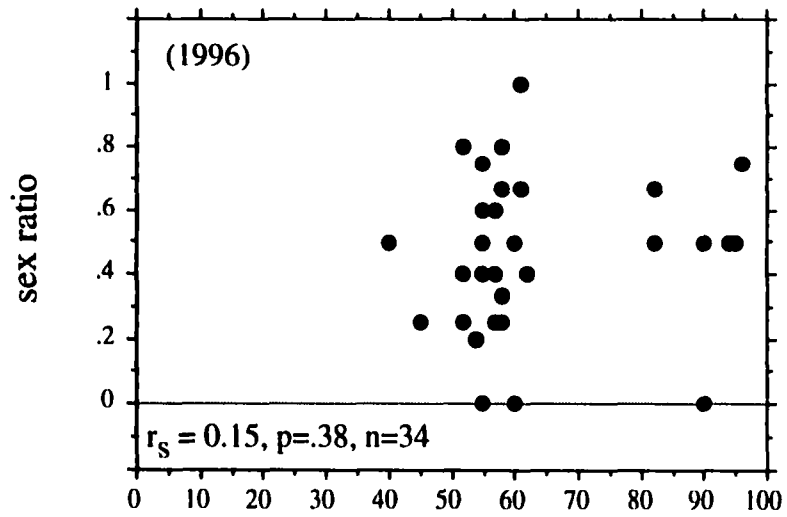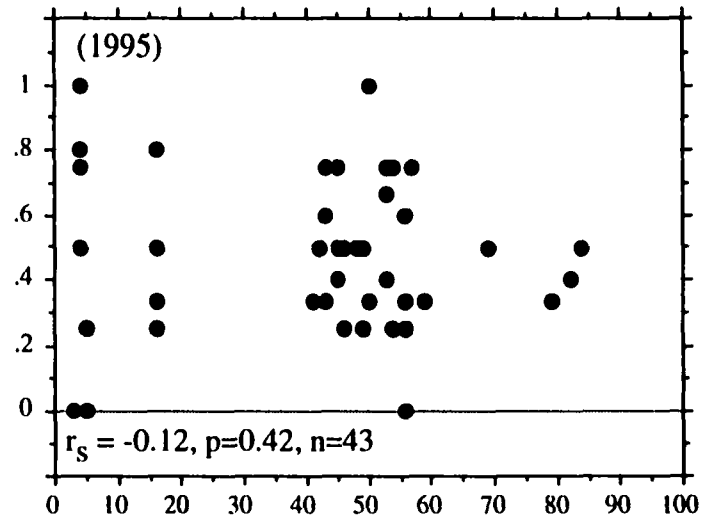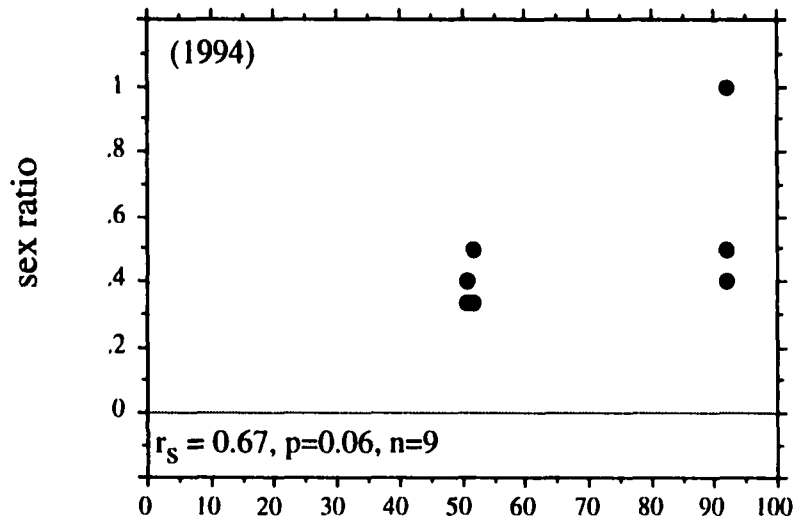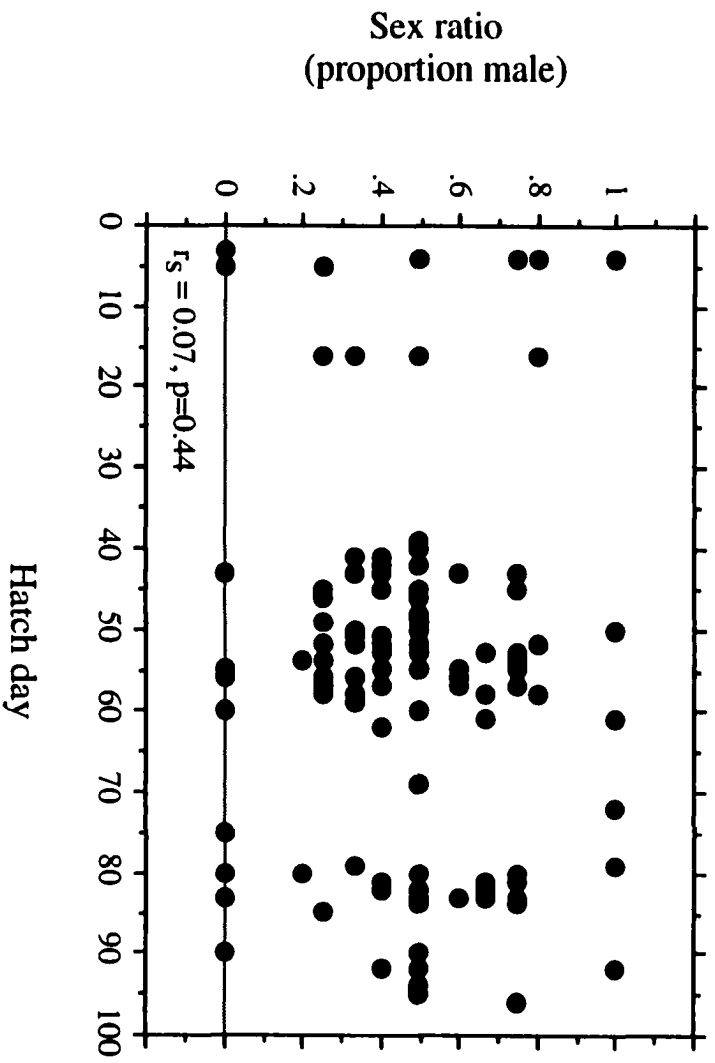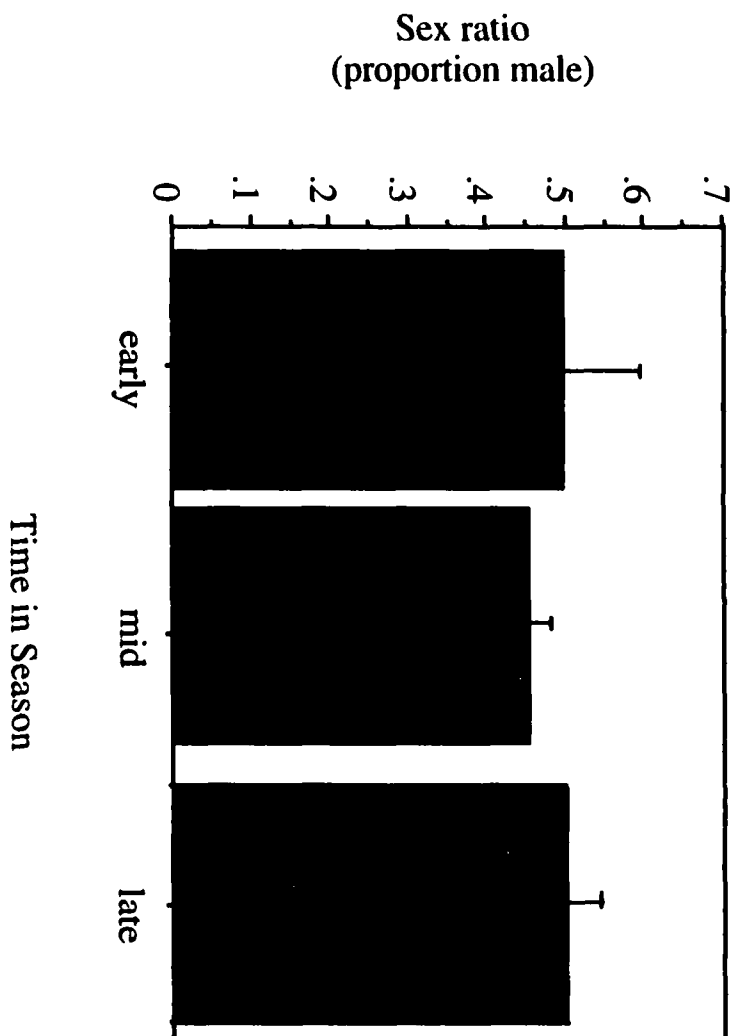
Brood sex ratios in relation to male badge size

in house sparrows, *Passer domesticus*

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Abstract. Recent studies have examined potential mechanisms for varying offspring sex ratio and have uncovered patterns of variation occurring under natural conditions. Adaptive variation in offspring sex ratio has been suggested in response to multiple factors including the attractiveness of one's mate. Burley (1986) found that zebra finch females mated to males whose attractiveness was artificially enhanced produced broods with a male-biased sex ratio. I examined brood sex ratio at hatching in house sparrows and whether that was related to the putative father's badge size, a trait reported to be correlated with mate attractiveness in this species. Thus, if badge size were heritable, females mated to large-badged males may increase their reproductive success by raising more sons than daughters. I used video-imaging analysis to measure male badge size from still photographs of field-trapped birds. Brood sex ratios at hatching were determined using flow cytometry to identify nestling gender. There was a positive correlation between brood sex ratio and the father's badge size with larger-badged males tending to have a greater proportion of sons than smaller-badged males. This agrees with the prediction that females mated to larger-badged males would bias the sex ratio in favor of sons, although whether they derive genetic or direct benefits from such biasing remains unclear.

Introduction

It has long been assumed that the primary sex ratios of vertebrates with chromosomal sex determination were constrained to 50:50 due to the random segregation of chromosomes during gamete production (Williams 1979; Charnov 1982). With the advance of modern molecular techniques and means of determining the sexes of very young offspring, there is increasing evidence that variation in the sex ratio does occur in vertebrates. This is particularly true in birds, where hatchlings could be reliably sexed early in the nestling period (prior to any brood reduction) only in the last few years. Evidence is fast accumulating that birds can and do vary the sex ratio of offspring produced at hatching (Ellegren et al. 1996; Svensson and Nilsson 1996; Komdeur et al. 1997).

There are many reasons why parents might vary the sex ratio at hatching. Previous studies have correlated sex ratio biases with sexual size dimorphism of nestlings (Olsen and Cockburn 1991; Bednarz and Hayden 1991), maternal age (Blank and Nolan 1983), hatch order (Ryder 1983), and time in season (Dijkstra et al. 1990). There have also been examples of variation of brood sex ratio according to the attractiveness of a mate (Burley 1986): in captive populations of zebra finches, *Poephila guttata*, there was a significant correlation between the attractiveness of the leg band color combination and the offspring sex ratio such that brood sex ratios at fledging were biased toward the sex of the attractive parent. Burley suggested that the bias in zebra finch sex ratios was due to parental manipulation after hatching because sex ratios of unreduced broods were not biased towards the attractive parent while nests in which all eggs hatched but some chick mortality occurred before fledging showed stronger sex ratio biases in the same direction as the overall trends. Burley further noted that brood reduction costs could limit the extent to which parents can bias offspring sex ratio affordably. Such costs could be avoided, however, if parents biased the sex ratio at

laying. If Burley's findings were generally applicable, one would expect to see either hatching or fledging sex ratio biases according to mate attractiveness in other species, where mate attractiveness is predicted by naturally occurring traits.

In house sparrows, *Passer domesticus*, Møller (1988) reported that females prefer males with larger badges: large-badged males are more likely to be mated, to defend territories with a greater number of nesting sites, and to elicit more copulation displays (as well as more intense displays) in estradiol-implanted females than males with smaller badges. In addition to its possible role in mate choice, Møller (1990) also suggested that badge size is a good predictor of male success in achieving copulations during both forced and unforced extra-pair mating attempts. Consequently, females mated to larger-badged males should bias the sex ratio of their offspring in favor of sons, assuming male badge size is a paternally conferred trait. I tested this prediction using house sparrow broods in which the badge size of the male at the nest was measured and the sexes of all hatchlings were determined.

Methods

House sparrows nesting in Norman, OK from 1994-1997 were used in this study. Birds readily use nestboxes and build dome nests beginning in mid-March. Nests were monitored for laying every 3-4 days. The first eggs generally hatched the first week of April and breeding continued through July, with birds laying 3-4 clutches per season. Birds were ground trapped and banded with US Fish and Wildlife aluminum leg bands and unique combinations of plastic, colored bands. Individual males were matched with their nestbox when they were resighted after release. Before release, males were photographed to obtain a standard picture of the black throat badge. Each male was held immobile on its back with the beak at a 90° angle to the ground. They were photographed with a .5 cm by .5 cm grid in the background that was later used to scale

the photos for image analysis. Each photograph was scanned into a computer and the perimeter of each badge digitized. Using the background grid for scale, area of the badge was calculated. Each photograph was digitized three times and the average of the three area calculations was used in the analysis. To eliminate potential scoring biases, photographs were coded by the birds' US Fish and Wildlife band numbers and matched to their nests and their brood sex ratios after the badges were measured. The date of capture of each male and the hatch date of the brood were recorded as the number of days after the first egg hatched in each season (8 April,1994; 7 April, 1995 7 April, 1996; and 12 April, 1997).

We used each male's brood at the time of his capture to sample the sex ratio of his offspring. Thus, only broods hatched in the same year that their putative father's badge was photographed were used in the analysis because we do not know how consistent badge size is between years. A small blood sample (< 50 µl) was collected from each chick in ACD anticoagulant by clipping a toenail; samples were collected when the chicks were 1-3 days old and stored at $4°$ C until analysis. I collected blood from a few broods ($<10\%$) when the chicks were older (9-10 days old), but only if no brood reduction had occurred. The sex of each chick in the study was identified using flow cytometry, following the protocol established in Tiersch et al. (1989). Sparrow blood samples were combined with an internal reference sample of catfish (*Ictalurus punctatus*) blood and stained with 0.5 mL of stain containing 0.1% sodium citrate, 0.1% Triton-X100, 25 µl RNAse and 25 µg propidium iodide. After staining, the cells were kept at room temperature and analyzed within 30 minutes.

The average DNA content per cell for each sample was calculated using the median channel of the fluorescence intensity peaks according to the formula: pg sparrow DNA = (B/C)*2.0 pg, where B=bird median channel, C=catfish median channel and 2.0 pg is the DNA content of catfish (Tiersch et al. 1989). DNA content for each unknown sparrow nestling was compared to known-sex samples run

concurrently. These known-sex samples were from breeding-plumage adults in the population that had been ground trapped and bled that same year. In a few cases of ambiguity in these comparisons, subjects were compared directly to their parents (if available) or with their siblings. If sex identification remained ambiguous, neither the nestling nor the brood it came from was used in the analyses. Once sex was identified for all nestlings, brood sex ratios were calculated as the proportion of the brood that was male. Sex ratios for 28 house sparrow broods were determined using these methods. Biases in the sex ratio from 0.5 were tested using a G-test with William's correction (Sokal and Rohlf 1981). All other analyses were carried out with JMP 3.2 (SAS Institute Inc.) and SAS 6.0 statistical software.

Results

There was no detectable effect of year on male badge size (Kruskal-Wallis $\chi^2 = 5.19$, p = 0.16), brood size ($\chi^2 = 0.675$, p = 0.88), number of male nestlings per brood ($\chi^2 = 2.045$, p = 0.56) or sex ratio ($\chi^2 = 3.11$, p = 0.37). Accordingly, data from all four years of this study were combined for the remainder of the analyses. The mean sex ratio (proportion of male nestlings) was 0.46, which did not differ from parity ($G_{adj} = 0.727$, p = 0.39).

Badge size, which ranged from 205 mm^2 to 466 mm^2 with a mean of 331.28 mm$^2 \pm 76.16$ (mean $\pm$ s.d.), was not correlated with capture date for males (r = 0.11, p = 0.56) (Figure 1). Brood size ranged from 3 to 5 chicks. Data for brood sex ratios and the number of males per brood were not normally distributed, so non-parametric tests were used for all analyses. Brood size was not correlated with male badge size ($r_s = -0.04$, p = 0.83); thus larger-badged males did not have larger broods than small-badged males. Proportion of sons per brood was higher for males with larger badges ($r_s = 0.40$, p = 0.03) (Figure 2) as was the number of male hatchlings produced

($r_s = 0.37$, $p = 0.05$) with larger-badged males having a greater number of sons. The proportion of males in a brood was not correlated to hatch day for nests, either in this study ($r_s = 0.29$, $p = 0.11$) or in a larger sample of nests from this population (Voltura, chapter 1).

Discussion

The results showed a significant, positive correlation between the attending male's badge size and the proportion of offspring that were male. Apparently, female house sparrows mated to large-badged males tend to bias their brood's sex ratios toward production of sons. This finding is consistent with that of Burley's (1986) results for fledgling zebra finches when parents had been given artificial ornaments. A relationship between mate attractiveness and brood sex ratio has been shown in collared flycatchers, *Ficedula albicollis*, as well (Ellegren et al. 1996). In that species, the size of a male's white forehead patch was positively correlated with the proportion of sons in his brood. Male forehead patch size is both heritable and condition-dependent in collared flycatchers. Ellegren et al. (1996) found that males that raised experimentally enlarged broods in one season had smaller forehead patches and fewer sons the following year when compared to males that had raised experimentally reduced broods the previous year. Similarly, brood sex ratio in blue tits, *Parus caeruleus*, has been shown to be related to male condition. Males that survived until the following breeding season had broods with a more highly male biased sex ratio than did males not surviving the following winter (Svensson and Nilsson 1996).

Male badge size in house sparrows may be condition- and/or age-dependent. Yearling males have significantly smaller badges than older males, even controlling for body weight and tarsus length (Veiga 1993). There is also some evidence for a nutritional component to badge size (Veiga and Puerta 1996): in the field, juveniles had

smaller badges than adult males, but when fed ad libitum in captivity, these badge size differences disappeared. Other evidence from captive birds shows that males that used more blood proteins during molt (a condition negatively correlated with fat reserves) produce smaller badges (Veiga and Puerta 1996). If male badge size is a signal of condition, females may use this to assess mates and adjust brood sex ratios accordingly. Alternatively, if male badge size increases with age in house sparrows, larger badges may signal higher survivorship prospects. Females mated to large-badged males may gain genetic benefits from producing more sons if the fitnesses of sons from large-badged males are greater than the fitnesses of daughters (i.e. if better body condition and good genes have a greater impact on male reproductive success than on female reproductive success). Alternatively, Møller (1989) has suggested a genetic component to badge size, based on a positive father-son regression and having found no relationship between badge size and condition. If badge size were heritable, females mated to larger-badged males might profit from biasing broods to favor sons because male offspring will inherit the attractive trait.

The relative importance of environment versus genes for male badge size is unclear for house sparrows and for the forehead patch of collared flycatchers (Ellegren et al. 1996). If females are using these male traits solely as a genetic indicator of mate quality, one would expect females to bias brood sex ratio irrespective of any contributions their mate makes to parental care. On the other hand, if females are using male traits as honest signals of condition, these traits may indicate that male's potential for providing quality care to the offspring. In house sparrows, larger-badged males do tend to have higher feeding rates (proportion of feeds per chick by the male) than smaller-badged males (Voltura, chapter 3). Females may be using badge size as an indicator of a mate's ability to care for young and thus gaining direct benefits from his increased paternal contribution. Experimental results indicate though that parents do not adjust their feeding rates to broods with extreme biases in sex ratio (Voltura,

Chapter 4); thus broods with a male-biased sex ratio do not necessarily require increased parental effort. But, if the fitness of sons is more affected by direct benefits from the male than the fitness of daughters, that might explain why large-badged males not only have more sons, but also feed proportionately more than smaller-badged males. If the amount of food provided by parents influences plumage characteristics of juveniles, such as badge size, but not necessarily fecundity or survivorship, then the level of care during the nestling period may be more influential for male fitness than for female fitness.

While I have shown that the sex ratio at hatching is related to male badge size, the fledging sex ratios are unknown for most of these nests. Many were part of other experiments and fledgling success may have been influenced by treatment effects. However, there is no evidence from experimental nests that sex-biased mortality is occurring before fledging (Voltura, chapter 4). If so, then fledging sex ratios would not be much different from sex ratios at hatching. Thus, it appears that females are biasing the sex ratio at hatching and not utilizing brood reduction to achieve biased brood sex ratios. The mechanism for the production of biased sex ratios at hatching is still unknown for birds. It has been suggested that parents might bias the sex of the first egg laid, either through selective resorption or abandonment of eggs, and then leave the sex of the remaining eggs up to chance to avoid increased hatching asynchrony (Emlen 1997). Other suggestions have included differential developmental rates of egg follicles (Ankney 1982; Krackow 1995), and, in other taxa, differential mortality of embryos (Krackow 1995) and meiotic drive mechanisms (Wilkinson et al. 1998)

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Figure legends

Figure 1   Frequency distribution of male badge size for the 28 house sparrows in this study.

Figure 2.   Male badge size correlated with brood sex ratio for house sparrows (n=28).

Badge size (mm^2)

Sex ratio
(proportion of males)

Badge size (mm²)

$r_s=0.40$, p=0.03, n=28
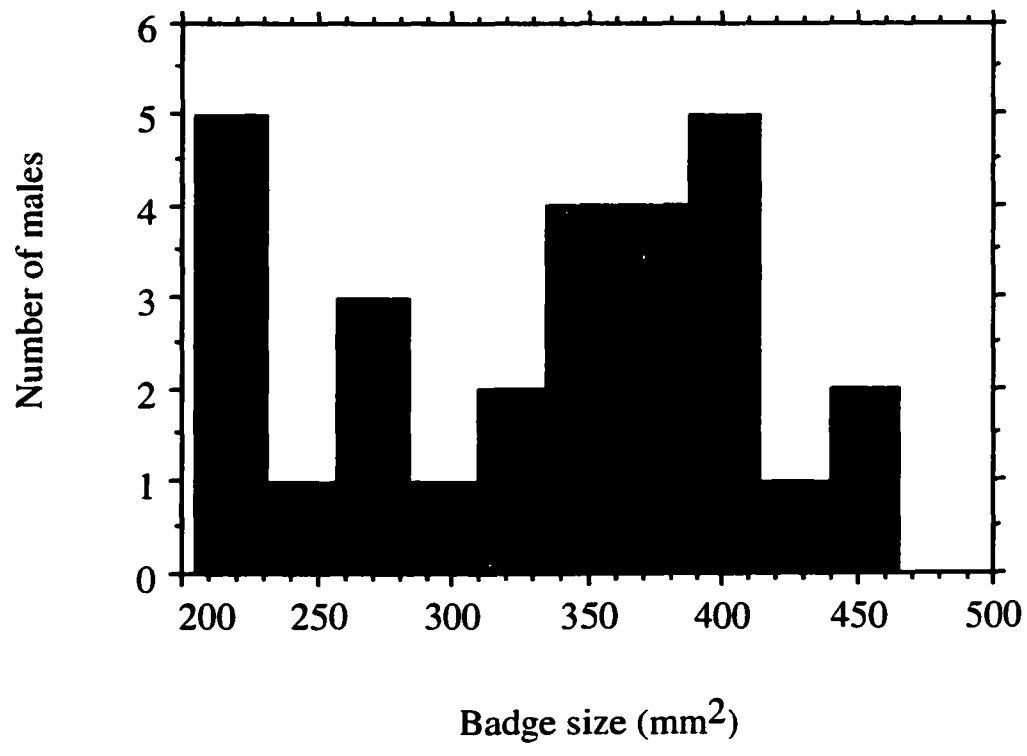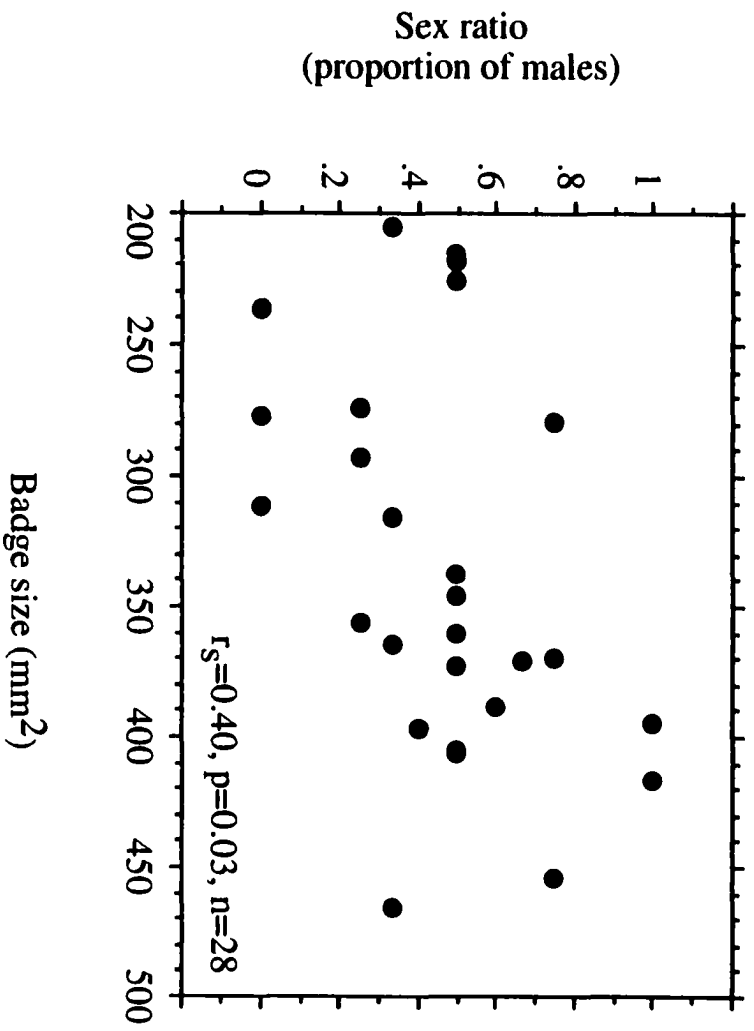
Parental feeding rates in house sparrows, *Passer domesticus*: are larger-badged males better fathers?

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Abstract. Sexual selection theory predicts that females may choose mates to gain benefits for themselves and/or their offspring. In several avian species, female mate choice apparently confers genetic benefits on the offspring; in other species, preferred males provide material benefits that may increase the probability of survival for the female and/or offspring. In many cases, female birds appear to base their choice of a mate on male plumage characteristics. These plumage characteristics may act as a signal of genetic quality or as an indicator of good health that reflects foraging ability or territory quality, and thus the potential to provide food for nestlings. Female house sparrows, *Passer domesticus*, reportedly prefer males with larger, black throat badges and we examined whether they gain material benefits form this preference. House sparrow nests where the male had been trapped and banded were observed for one hour on at least five days during the peak growth period of nestlings. Feeding rates for males, measured as the proportion of total feeds per chick made by the male, were positively correlated with male badge size, the result of the tendency for large-badged males to have higher absolute food delivery rates than small-badged males and for the mates of large-badged males to have slightly lower food delivery rates than the mates of small-badged males. also fledged. A greater proportion of chicks fledged from the nests of larger-badged males. These results support the hypothesis that females mated to large-badged males gain direct benefits through increased paternal contributions in feeding, which translates to increased survival of offspring, at least to fledging.

Introduction

Sexual selection theory predicts that females choose mates to gain direct or indirect benefits for themselves and their offspring. Indirect benefits might influence female mate choice if females choose a mate of high genetic quality and thus increase the survival and reproductive success of their offspring. Direct, or non-genetic, benefits that females might gain from mate choice include: increased fecundity (e.g., because of food provided by her mate during courtship or incubation), increased parental care of offspring by her mate, access to better food sources on the male's territory, and a potentially lower risk of predation or lower levels of harassment from other males (Andersson 1994).

There have been several theories dealing with female choice and male parental quality that make opposing predictions regarding the amount of parental care provided by attractive males. The "sexy-son" hypothesis (Weatherhead and Robertson 1979) predicts that attractive males contribute less than less attractive males to parental care of offspring and thus may decrease the number of young a female produces, but the sons of females mated to attractive males inherit the attractive trait from their father and thus have higher reproductive success than sons of less attractive males. The differential-allocation hypothesis (Burley 1986) makes a similar prediction that more attractive males will have lower parental expenditure than less attractive males because more attractive males are favored to allocate more time to seeking additional mating opportunities. Alternatively, several 'good-parent' models have predicted that females are selected to choose males that increase their fecundity (Kirkpatrick 1985) and that traits that females find more attractive can evolve as honest signals of male parental quality (Hoelzer 1989). We examined the hypothesis that male house sparrows, *Passer domesticus*, that are reportedly more attractive to females, provide increased parental care for their offspring, thus increasing offspring survival.

In birds, female mate choice is often based on exaggerated male secondary sexual characteristics. If females choose mates based on conspicuous male plumage traits or behavioral characteristics such as song, these traits may indicate high genetic quality of the male or they may signal that a male is in better condition and thus able to provide more care for her offspring. Empirical evidence for genetic benefits for female choice in birds has been found in house finches *Carpodacus mexicanus* (Hill 1991), great tits, *Parus major* (Norris 1993), and great reed warblers, *Acrocephalus arundinaceus* (Hasselquist et al. 1996). Female house finches were shown to gain direct benefits from their mate choice as a result of higher feeding rates by brighter males (Hill 1991). Direct benefits for female choice through increased paternal contributions to parental care have also been found in pied flycatchers, *Ficedula hypoleuca* (Saetre et al. 1995), American kestrels, *Falco sparverius* (Wiehn 1996), and northern cardinals, *Cardinalis cardinalis* (Linville et al. 1997).

In house sparrows, males have conspicuous black badges on their throats. It has been reported that female house sparrows prefer to mate with males with larger badges (Møller 1988). Larger badged males are said to participate in communal displays more frequently than smaller badged males, and have higher rates of copulation, both within-pair and extra-pair. Large-badged males also are more likely to be mated and to have territories of better quality than small-badged males (Møller 1988). Females may be choosing males with larger badges because of the potential genetic benefits to her offspring if the offspring of larger badged males are more likely to survive and have higher reproductive success due to some genetic component inherited from their father. Alternatively, females may gain direct benefits from choosing large-badged males if those males provide increased parental care to offspring when compared to small-badged males. By observing the parental behavior of males of known badge size and examining feeding rates and nestling survival in relation to male badge size, we tested the hypothesis that males with large badges would contribute more to nestling care than smaller-badged males.

Methods

Four nestbox populations of house sparrows in Norman, OK were studied from 1994-1997. Birds began building nests in mid-March and nestboxes were checked every 3-4 days to monitor laying. The first clutches hatched in early April and birds continued breeding through the summer, laying 3-4 clutches over a single breeding season. We captured birds nesting in these boxes with mist nets, ground traps or wire corridor traps and banded them with US Fish and Wildlife aluminum leg bands and unique combinations of plastic, colored bands. Before release, we photographed males to obtain a standard picture of the black throat badge. Males were held immobile with his beak pointing upwards, at a 90° angle to the ground. They were photographed with a .5 cm by .5 cm grid in the background which was later used to scale the photos. Individual males were matched with their nestbox when they were resighted and identified after release. Later, we scanned the photographs into a computer and digitized the perimeter of the badge. Using the background grid to scale each photo, the area of the badge was calculated. Each photograph was digitized three times and the average of the three area measurements was used in the analysis. To eliminate potential scoring biases, we coded the photographs by the birds' US Fish and Wildlife band numbers and matched males to their nests after the badges were measured. The date of capture and the hatch date of the brood were measured as the number of days after the first egg hatched in the population. This date was 8 April in 1994, 7 April in both 1995 and 1996, and 12 April in 1997.

We sampled feeding rates for each male during the breeding cycle at the time of his capture. Thus, only feeding rates measured in the same year that a male's badge was photographed were used in the analysis because we do not know how consistent badge size is between years. Focal nest observations were made over days 3 through 11 of the nestling period (where day 0 is the day hatching began). We recorded feeding rates at each nest, distinguishing between maternal and paternal feeding visits. At least

five, 60 minute focal nest observation samples were collected for each nest over the 9

day period. Observations were staggered throughout the day such that all five samples

were not made during either the morning or afternoon time periods. For 13 nests we

quantified the type of food delivered in each visit as large (>3/4 inch [19mm] in length),

medium (3/4 - 1/2 inch [12.7-19 mm] in length), small (<1/2 inch [12.7 mm] in

length), or seed and bread. In some cases we were unable to classify size of prey

delivered for each visit to the nest so we calculated prey size variables as the proportion

of visits where prey size was identified where either small, medium or large prey items

were delivered to nestlings. These proportions were not normally distributed and non-

parametric correlations were used to analyze the results.

We weighed chicks on day 11 and banded them with U.S. Fish and Wildlife

Service aluminum bands and colored, plastic leg bands. Nests were checked after day

17 to identify any chicks that had not fledged and were dead in the nestbox. Mean

chick mass and the proportion of chicks fledged were not normally distributed either

and non-parametric correlations were used for those analyses as well.

Statistical analyses were performed using JMP 3.2 (SAS Institute Inc.) and

SAS 6.0 statistical software.

Results

Badge size ranged from 177 mm^2 to 693 mm^2 and was not correlated with date of

capture of the male ($r_s = 0.21$, $p = 0.28$). The proportion of feeding done by

individual males ranged from 0.066 to 0.688. There were no differences among years

of this study in male feeding visits/nestling/hour (Kruskal-Wallis $\chi^2 = 3.41$, $p = 0.33$),

female feeding visits/nestling/hour ($\chi^2 = 4.50$, $p = 0.22$), nor in the proportion of

feeds by fathers ($\chi^2 = 0.74$, $p = 0.86$) and data for all 4 years were combined for the

remainder of the analyses.

In general, female feeding rates were higher than male feeding rates for nests in this study (t = 2.162, p = 0.035, n = 27); females made an average of 2.82 feeding visits per nestling per hour and males made only 2.12 visits per nestling per hour. Feeding rates varied with time in season: mean number of feeds per nestling was negatively correlated with hatch day, both for mothers (r = -0.41, p = 0.02, n = 27) and for fathers (r = -0.45, p = 0.03, n = 27), though the proportion of total feeds per nestling made by fathers was not (r = 0.02, p = 0.91, n = 27). Male and female feeding rates were positively correlated with each other (r = 0.68, p = 0.006, n = 27) (Figure 1), and the relationship remained positive with the effects of hatch day partialed out ($r_{xy \cdot z}$ = 0.40, p = 0.04, n = 27).

The total feeds per nestling for each nest, after controlling for hatch day, was unrelated to male badge size ($r_{xy \cdot z}$ = -0.02, p = 0.94, n = 27). However, the proportion of feeds made by the male at a nest was positively correlated with the size of his badge (r = 0.38, p = 0.05, n = 27) (Figure 2). Two factors led to this effect of badge size on proportion of feeding by the male: the mean feeds per nestling for fathers with hatch day controlled for was slightly positively correlated with badge size ($r_{xy \cdot z}$ = 0.17, p = 0.42, n = 27) while the mean female feeds per nestling was slightly negatively correlated with her mate's badge size ($r_{xy \cdot z}$ = -0.13, p = 0.50, n = 27).

The mean mass of nestlings at day 11 of the nestling period increases as the season progresses (r = 0.38, p = 0.07, n = 24) and was not correlated with male badge size once hatch day was controlled for ($r_{S\,xy \cdot z}$ = -0.07, p = 0.73, n = 24). Although larger-badged males did not have broods with heavier nestlings, they did fledge a greater proportion of the chicks that hatched in their nest (r_S = 0.43, p = 0.03, n = 27) (Figure 3).

For 13 nests with data on size of prey delivered to the nest, the proportion of feeds by either fathers or mothers that were large prey items was not correlated with male badge size (Table 1). For males, though, the proportion of feeds that were

medium-sized prey was negatively correlated with male badge size and the proportion of small prey items fed increased with increasing badge size (Table 1).

Discussion

Larger-badged male house sparrows make proportionately more feeding trips than smaller-badged males and fledge a higher proportion of the young in their nests. It has recently been reported that nest defense is also related to badge size in this species: large-badged males have more intense responses to mounted predators (Reyer et al. 1998). The combination of proportionately higher feeding rates and increased nest defense indicates that females may be gaining direct benefits from choosing large-badged males and agrees with other studies showing that more attractive males are better providers. In northern cardinals, the proportion of feeding done by each parent was positively correlated to the brightness of their plumage coloration (Linville et al. 1997). In American kestrels, males with bright plumage and narrow subterminal tail bands (traits indicative of older birds) had higher provisioning rates and produced more fledglings than dull males with wider subterminal tail bands (Wiehn 1997).

Although nestlings from the broods of large-badged males are not receiving more total feeding visits, those males are making proportionately more visits to the nest than smaller-badged males. Even though large-badged males may be feeding more small prey items and fewer medium sized prey items to their offspring, their feeding rates may allow their mates to spend more time brooding than females mated to small-badged males. In polygynous house wrens, *Troglodytes aedon*, males give little or no help to secondary mates (Johnson et al. 1993). Those secondary females have lower reproductive success than primary females and this has been attributed to the competing demands on a single parent to increase feeding rates and still maintain brooding times that keep nestling metabolic rates at normal levels as well as protect nestlings from

intruders (Johnson and Kermott 1993). It is possible that female house sparrows mated to large-badged males have more time to spend brooding than females mated to small-badged males and this may be one reason broods with larger-badged males in attendance fledge proportionately more young.

The results for paternal feeding rates are the opposite of those predicted and tested by Burley (1988). She hypothesized that attractive males should allocate more effort towards extra-pair matings and polygynous matings and thus decrease investment in the current brood. Conversely, unattractive mates could enhance their fitness by increasing their contributions to parental care and thus acquire and retain mates as well as increase the quality of their offspring. In captive populations of zebra finches, *Poephila guttata*, males with less attractive leg band combinations did have higher rates of parental expenditure than more attractive males. More attractive males were more likely to be polygynous and thus had lower levels of parental expenditure than less-attractive males. Low levels of polygyny generally occur in some house sparrow populations, including the ones in this study, but none of the males used in this study were polygynous at the time they were sampled and the relationship between polygyny and parental effort in relation to badge size is unknown in house sparrows. Møller (1990) found that large-badged males have higher rates of copulation and participate in communal displays more often than smaller-badged males but in our study, larger-badged males apparently do not decrease the effort allocated to the care of nestlings.

Our results from house sparrows support the hypothesis that females use male indicator traits to assess the quality of their mate as a provider for offspring. But how might badge size evolve as a signal of a male's ability to care for offspring? Wolf et al. (1997) modeled the evolution of indicator traits for parental quality, showing how a phenotypic correlation could evolve between a male trait and the level of parental investment he provides. In their model, indirect genetic effects lead to male traits that act as honest signals of the amount or quality of care that male will provide to

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offspring. If maternal and paternal effects (i.e. the amount of parental investment that mothers and fathers provide during the period of parental care) are heritable and effect the expression of an indicator trait in male offspring, then a phenotypic correlation can evolve between the amount of paternal care a male will provide and the expression of an indicator trait. The degree to which the male trait and the amount of parental care he provides are correlated determines the honesty of the trait: the stronger the correlation, the more reliable the signal (Wolf et al. 1997). Badge size in house sparrows has been claimed to be heritable, based on a significant father-son regression (Møller 1989), but there are also studies that show a nutritional component to badge size (Veiga and Puerta 1996). If phenotypic expression of badge size is related to the quality of care offspring receive from the father during the nestling period, then a correlation between male badge size and the amount of parental effort he will put forth can evolve. According to the model by Wolf et al. (1997), genetic covariance between male and female investment may result in maternal effects that influence the expression of the indicator trait in sons and thus paternal investment by those sons will be similar to the level of investment by their mother. In house sparrows, male and female feeding rates are correlated, thus there could be genetic covariance between the components inherited from the mother and the father and this too would support the hypothesis that badge size is an honest indicator of male parental quality.

Badge size is not related to testosterone levels in the male house sparrow (Owens and Short 1995), but may be related to age as well as to indices of body condition in free-living individuals (Veiga 1993; Veiga and Puerta 1996; but see Møller 1989; Lifjeld 1994). After the fall moult male badge size is larger for 2-year old males than for juvenile or 1-year old males, but does not vary with age among males older than 2-years of age (Veiga 1993). If larger-badged males tend generally to be older and more experienced breeders, the correlation between badge size and paternal feeding may be a side effect of age. In red-winged blackbirds, *Agelaius phoeniceus*, older

males have larger song repertoires and lengthier courtships than younger males (Searcy and Yasukawa 1981) and males with these attributes have better territories and are preferred by females. These more experienced males also feed nestlings at higher rates than younger males (Searcy and Yasukawa 1981; Yasukawa et al. 1990) and the number of fledglings produced per nest correlates with feeding rates (Yasukawa et al. 1990).

While we have shown that females gain directly from pairing with large-badged males, this does not rule out the possibility that they might also gain indirect benefits from this choice. It is possible that the higher proportion of young fledged from the nests of larger-badged males is due to some genetic quality inherited from the father. Without paternity analyses and cross-fostering experiments to separate the genetic effects from maternal and paternal effects, we are unable to make any conclusions at this time. But, there is no evidence that male badge size effects adult survivorship, nor is male badge size related to paternity (R. Whitekiller, unpublished data). Another study has shown that female house sparrows mated to large-badged males produce more sons than females mated to small-badged males (Voltura, chapter 2). If male badge size is heritable, as has been suggested by Møller (1989), then the sons of large-badged males would inherit this favorable trait and thus have increased reproductive success when compared to the sons of small-badged males. This correlation between male badge size and brood sex ratio may thus be related to the genetic quality of the male but his parental effort may influence the expression of that trait in offspring. Finally, some studies have indicated that badge size is condition-dependent, especially among juveniles and males in their first breeding season (Veiga 1993; Veiga and Puerta 1996), so higher paternal feeding contribution, combined with the heritability of badge size, could result in larger-badged males having more sons with larger badges.

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Table 1. The partial correlations of male badge size with the proportion of feeds of a given prey size controlling for the effects of hatch day (n=13).

Parent

Prey size	mother	father
small	$r_{S\ xy \cdot z} = 0.56$, p = 0.06	$r_{S\ xy \cdot z} = 0.64$, p = 0.02*
medium	$r_{S\ xy \cdot z} = -0.31$, p = 0.33	$r_{S\ xy \cdot z} = -0.78$, p = 0.002*
large	$r_{S\ xy \cdot z} = -0.34$, p = 0.29	$r_{S\ xy \cdot z} = -0.20$, p = 0.53

Figure legends

Figure 1.   The correlation between maternal and paternal feeding visits/nestling/hour (n=27).

Figure 2.   The correlation between the proportion of feeds by the male at a nest and his badge size (n=27).

Figure 3.   The proportion of the chicks that hatched in a nest that fledged correlated with male badge size (n=27).

Proportion of feeds
by the male

Badge size (mm²)

r=0.38, p=0.05, n=27

Proportion of the
brood that fledged

Badge size (mm^2)

$r_s = 0.43$, p=0.03, n=27

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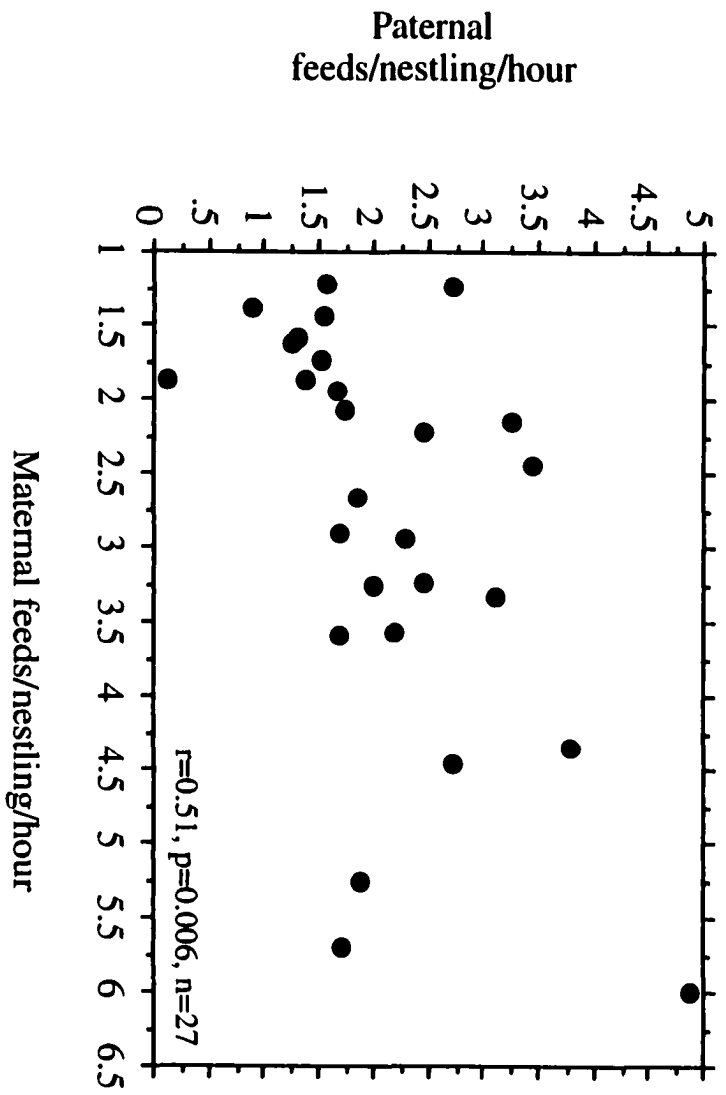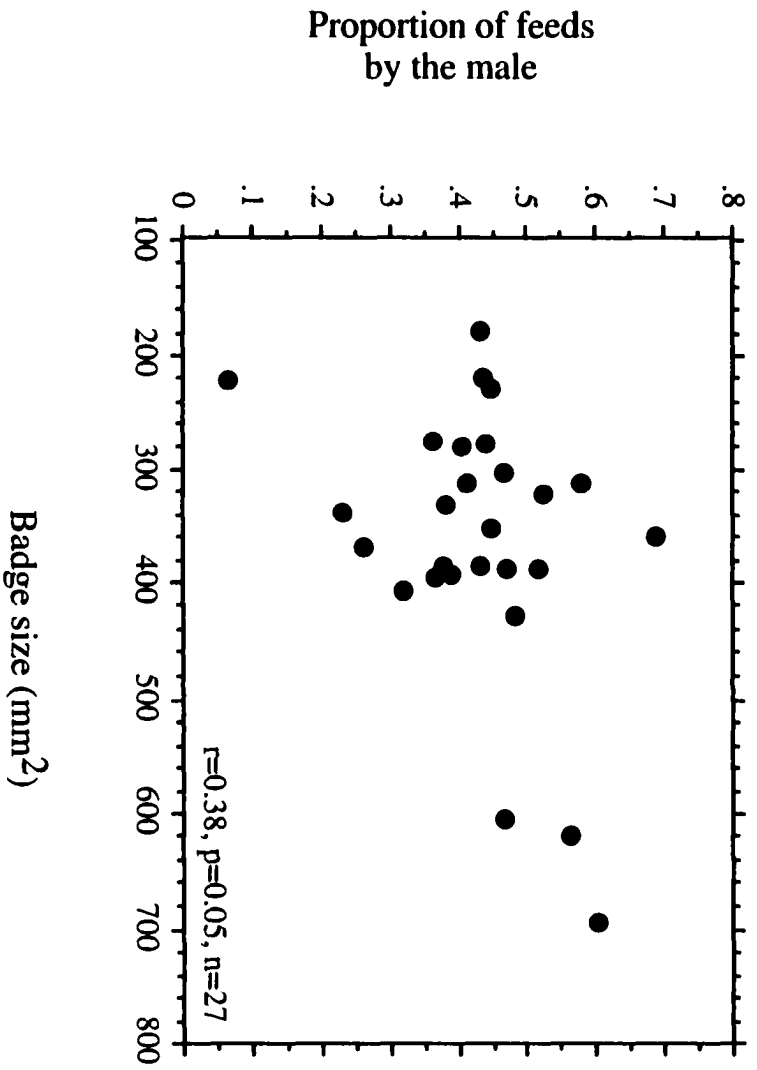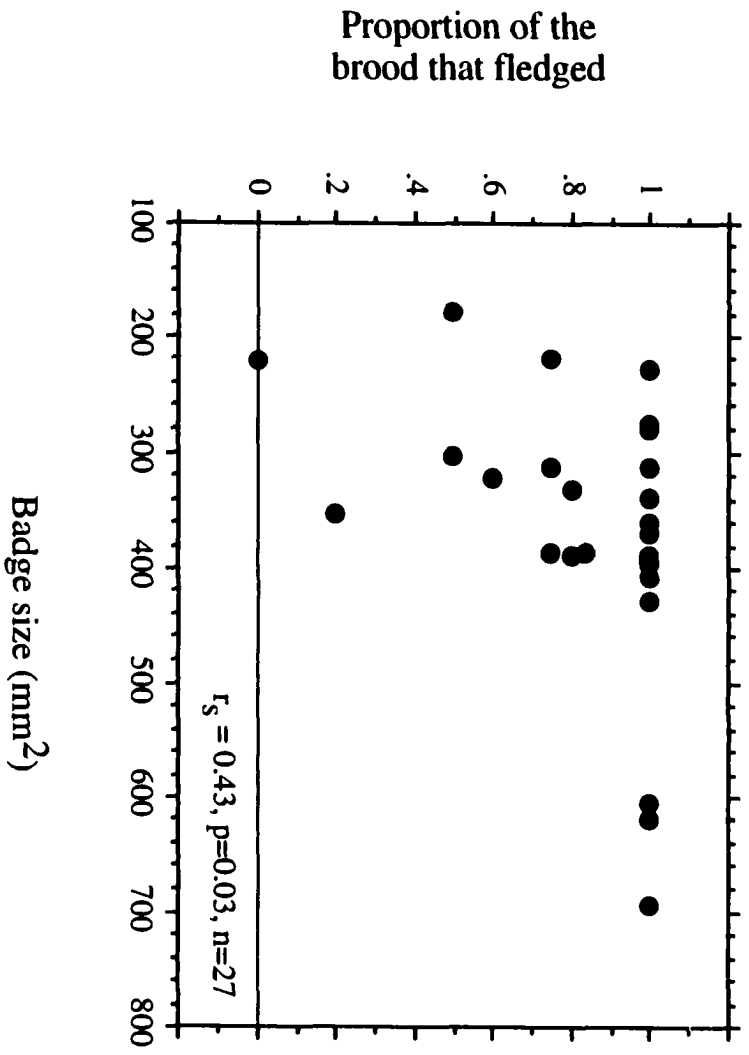
Parental investment in house sparrows, *Passer domesticus*: an experimental manipulation of brood sex ratio

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Abstract. The influence of offspring gender on provisioning rates and nestling growth was examined in house sparrows by experimental manipulation of brood sex ratio. I sexed nestlings using flow cytometry and cross-fostered 3- to 4-day old nestlings to create single-sex experimental nests and mixed-sex control nests. I measured parental feeding rates and nestling growth rates throughout the nestling period. The maternal and paternal feeding rates did not differ among the treatment groups, nor did the combined feeding rates. There were also no significant differences in growth rates or final weights for the three nest types (all-male, all-female and mixed-sex). These results indicate that although ecological and behavioral differences exist between male and female offspring, these differences do not necessarily lead to differences in parental care during the nestling period. Other work has shown that house sparrows produce biased sex ratios: females prefer males with larger badges and females mated to larger-badged males produce more sons than females mated to males with smaller badges. In house sparrows, differential sex allocation may be expressed in biased brood sex ratios rather than in differential parental care during the nestling period.

Introduction

Sex allocation is defined as the relative amounts of resources parents allocate to sons versus daughters. Therefore, sex allocation involves not only investment in the production of male and female embryos, but, in species with parental care, the costs associated with raising sons and daughters to independence. Differences in sex allocation are thus reflected in differences in offspring sex ratios and in differences in parental investment in offspring of each sex. Fisher's theory on adaptive sex ratios (1958) predicts that, overall, parents should invest equally in the two sexes. In cases where the sexes differ in the amount of investment necessary to achieve the same relative fitness level, many authors have hypothesized that selection will favor parents that bias allocation to maximize the return on their investment (Wilson and Pianka 1963; Trivers and Willard 1973; Charnov 1982; Stamps 1990; reviewed in Frank 1990). Charnov (1982) incorporated the idea of non-linear returns into the model and showed that departures from equal allocation to the two sexes could be favored if the marginal return on a given unit of parental investment differs for sons and daughters. Trivers and Willard (1973) hypothesized that differences in the variance in reproductive success of males and females could result in different marginal returns for the two sexes, thus favoring patterns of sex-biased provisioning of offspring. If minor differences in offspring quality have a greater impact on the reproductive success of one sex relative to the other, then parents in good condition may increase their fitness by investing more in the sex with higher variance.

Stamps (1990) suggested other factors that might lead to sex-biased provisioning in avian systems, including sexual size dimorphism, sibling competition, and developmental and behavioral differences between male and female nestlings. In species with little or no sexual dimorphism, sex-biased provisioning might occur if competition between the sexes takes place late in the dependent period or shortly after

independence is attained, when body size is still related to parental feeding rates. For example, dominance status might be based on sex in juvenile or winter feeding flocks and extra provisioning of individuals belonging to the subordinate sex might increase their chances of survival during the early stages of independence (Stamps 1990). Similarly, sex-biases in the timing or distance of dispersal could result in food being more important to the survival of the dispersive sex relative to the non-dispersive sex at this particular stage (Stamps 1990). Local resource competition has also been proposed as a source of differential cost of male and female offspring if one sex is more likely to disperse than the other (Gowaty 1993). Any sex-biased patterns of provisioning would influence the relative costs of raising sons and daughters, and so influence the reproductive potential of the parents.

It is also possible that differential parental investment patterns are not the result of selection on the parents to bias allocation but of selection on offspring of one sex to maximize growth during the early stages of development (Clutton-Brock 1991). If fledgling or juvenile condition is more important to the future reproductive success in one sex than the other, then selection pressures may influence offspring to behave in ways that result in differential allocation. In birds, the feeding patterns of parents are often correlated with nestling behavior and begging rates. In red-winged blackbirds, *Agelaius phoeniceus*, the parents do not selectively feed certain individuals; instead, it is the position in the nest and the height that individual reaches while begging that determine which nestlings are fed (Teather 1992). There is also evidence that parental feeding rates can vary between nests based on the overall brood sex ratios even though parents do not discriminate among individuals in those nests (Stamps et al. 1987). If selection acts on parents to bias allocation to the two sexes, one might expect that they would preferentially feed offspring of one sex more than the other, even within nests, but so far there are only two examples of gender discrimination by parents (Gowaty and Droge 1990). It has been suggested, though, that selective feeding of particular

offspring would slow overall food delivery to the nest; therefore, parents might adjust feeding rates to the entire brood based on the needs of the sex present in higher proportion (Stamps et al. 1987).

Although there are many reasons why avian parents might bias provisioning of sons and daughters, such biases have only been demonstrated in a few species. Eastern bluebird (*Sialia sialis*) fathers provision female-biased nests more than nests with sex ratios that are 50/50 or biased in favor of sons (Gowaty and Droge 1991). A similar pattern is seen in captive populations of budgerigars, *Melopsittacus undulatus*, with fathers also starting to feed daughter-biased nests earlier than other nests (Stamps et al. 1987). In neither case were female feeding levels correlated with brood sex ratios. In red-winged blackbirds, brood sex ratio influenced male as well as female feeding rates, with broods containing more sons receiving extra food (Yasukawa et al. 1990). This feeding bias was attributed to the higher food requirements of male nestlings due to their slightly larger size later in the nestling period.

In house sparrows, *Passer domesticus*, males are slightly larger than females by the later phase of the nestling period but females are more likely to disperse than males (Lowther 1979; Fleischer et al. 1984). Extra-pair paternity has been documented in house sparrows with a mean of 14% of nestlings sired by an extra-pair male (Wetton and Parkin 1991), and under certain circumstances males can be polygynous (Møller 1990; Veiga 1992). Therefore, male house sparrows may have a higher variance in reproductive success than females because some males can increase their reproductive success with extra-pair young and multiple mates. By extension of the Trivers and Willard hypothesis, it can be predicted that parents should increase their investment in the sex with the higher variance in reproductive success, not just by biasing the sex ratios but by biasing allocation to that sex during the period of parental care. Because male variance in reproductive success may be higher than female variance in reproductive success, and because male nestlings are slightly larger than females, I

hypothesized that house sparrow parents would invest more in male nestlings than in female nestlings. Alternatively, if dispersal patterns are influencing parental feeding rates, then parents might invest more in daughters, the sex more likely to disperse. If the amount of parental care influences success in dispersing, parents may have higher feeding rates to the dispersive sex. By experimentally manipulating nestlings to create single sex broods I looked for patterns of biased investment as indicators of the relative costs of offspring. If there are different costs of raising sons and daughters, these should be reflected in a divergence of parental feeding rates at male-only and female-only nests as well as divergences in growth rates and weights at fledging in the biased broods.

Methods

House sparrows nesting in four nest-box populations in Norman, OK, from 1995–1997 were used in this study. Birds readily use the boxes and begin building dome nests in them by mid-March. Nests were censused every 3–4 days to monitor laying. The first eggs generally hatched the first week of April and breeding continued through July, with individual females laying 3–4 clutches over a single season. For each set of experimental nests, 4 or 5 nests were chosen that hatched within one day of each other. The normal hatching interval is one day and by choosing nests that hatched only one day apart, I avoided increasing the variation in nestling size within a nest. A small blood sample (< 50 μl) was collected from each chick in ACD anticoagulant by clipping a claw; samples were collected when the chicks were 1-3 days old and stored at 4° C until analysis. The sex of each chick in the study was identified using flow cytometry, following the protocol established in Tiersch et al. (1989). Sparrow blood samples were combined with an internal reference sample of catfish (*Ictalurus punctatus*) blood and stained with 0.5 ml of a stain mixture composed of 0.1% sodium citrate, 0.1%

Triton-X100, 25 µl RNAse and 25 µg propidium iodide. The stained cells were kept at room temperature and analyzed within 30 min.

The average DNA content per cell for each sample was calculated using the median channel of the fluorescence intensity peaks according to the formula: pg sparrow DNA = (B/C)*2.0 pg, where B=bird median channel, C=catfish median channel and 2.0 pg is the DNA content of catfish (Tiersch et al. 1989). DNA content for each sparrow nestling was compared to known-sex samples run concurrently. These known-sex samples were from adults (which are sexually dimorphic) in this same population that had been captured in walk-in wire traps and bled that same year. If there was ambiguity in these comparisons, birds were compared directly to their parents (if available) or with their siblings. If sex was still ambiguous, the chick was excluded from the experiment.

Once sex was identified, I cross-fostered nestlings on day 4 of the nestling period to create three synchronous experimental nests, one composed exclusively of male nestlings (hereafter all-male), one all-female and one mixed-sex control nest. Original brood sizes were maintained so that parents were not raising reduced or enlarged broods. For the control nest, the original sex ratio was also maintained so parents were raising the same brood composition as the one they had produced, though none of the chicks were their own offspring. Parental feeding rates were recorded for one hour periods on at least 5 days during the nestling period, beginning on day 4 (with day 0 as the day the first chicks hatched in the nest). Observations were made between 0700 and 1700 hours and were made at different times each day so all samples were not made at either early or late times of the day for each nest. Chicks were weighed every other day and on day 11 were weighed for the last time and banded with U.S. Fish and Wildlife aluminum leg bands plus a unique combination of plastic, color leg bands.

This experiment was designed as a randomized block design to control for seasonal effects on feeding rates and growth rates. The number of feeding visits per

hour decreases as the season progresses and other factors such as temperature and rainfall can influence the relative amount of time parents spend feeding and brooding young nestlings. In this design, those factors will affect each nest within a block equally and thus the experimental nests within a block are more similar to each other than to nests in other blocks. In situations where one parent deserted within a day of the experimental manipulation, the entire set of three nests was omitted from the analyses. Also, several nests were lost to predation and those nests plus the block to which they belonged were removed from the data set. In addition, to avoid any effect that experimental manipulations might have on future reproductive effort, nests that had been used in previous manipulations were not re-used. All data were analyzed with repeated measures ANOVA. There was a total of 16 blocks (48 nests) used in each analysis. All statistical analyses were performed using JMP 3.2 (SAS Institute Inc.) or SAS 6.0 statistical software.

Results

The delivery rates of individual parents did not differ across all-male, all-female and mixed-sex broods (male feeding visits/nestling/hour: repeated measures $F_{2,14} = 0.796$, $p = 0.47$; female feeding visits/nestling/hour: $F_{2,14} = 0.727$, $p = 0.50$) nor was there any interaction between the gender of the parent feeding and the treatment ($F_{2,14} = 1.168$, $p = 0.34$) (Figure 1). That pattern held after male and female feeding rates were combined ($F_{2,14} = 0.062$, $p = 0.94$). Across all nests, female parents fed at slightly higher rates than males, but not significantly ($F_{1,15} = 3.853$, $p = 0.07$). The mean number of feeding visits/nestling/hour for mothers was 3.25 ± 1.13 (mean $\pm$ s.d.) and the mean for fathers was 2.68 ± 1.11. Moreover, the overall proportion of feeds by male parents was not affected by treatment ($F_{2,14} = 1.147$, $p = 0.35$) (Figure 2), though males delivered a slightly higher proportion of feeds at the all-male nests relative

to all-female nests ($F_{1,15} = 2.31$, p = 0.14). The mean number of feeding visits per hour per nestling was not correlated with brood size for fathers (r = 0.07, p = 0.63) or mothers (r = -0.18, p = 0.21), thus differences in brood size within blocks did not influence the results for feeding rates. Final weights did not differ among the three brood types ($F_{2,14} = 0.267$, p = 0.77), nor was there a difference in growth rates over the experimental period ($F_{2,14} = 0.16$, p = 0.85) (Figure 3). Power calculations for all tests ranged between 0.65 and 0.85 for estimated effect sizes and for the sample sizes in these analyses.

Within the control nests I also looked for differences in feeding rates based on sex ratio. Because I maintained the original sex ratios of control broods at the time the manipulations were done (day 4 of the nestling period), not all control broods had sex ratios of 0.5. There was no relationship between sex ratio of the brood during the experiment and the feeding rates of the mother ($r_s = -0.03$, p = 0.91) (Figure 4a). For males at the nest, however, there was a trend for the mean feeds/nestling/hour to increase with the proportion of sons in the nest ($r_s = 0.44$, p = 0.09) (Figure 4b) and consequently, the total amount of feeds/nestling/hour delivered to the nest also tended to increase with the sex ratio ($r_s = 0.40$, p = 0.13) (Figure 4c). The proportion of feeds/nestling/hour made by the male was not correlated to the sex ratio of the brood, although the trend was the same, the proportion of feeds by the male increased with an increase in the proportion of sons in the brood ($r_s = 0.31$, p = 0.23) (Figure 4d). There was no difference in nestling mass at day 11 (paired-t = -0.29, p = 0.77) or in growth rates (paired-t = 0.17, p = 0.89) when male and female nestlings within the control nests were compared, nor did nestling mass ($r_s = 0.02$, p = 0.93) or growth rates ($r_s = -0.16$, p = 0.55) correlate with brood sex ratio.

It is possible that parents do not adjust their level of parental investment in response to the manipulation of the sex ratio and that they might set their feeding rates based on the sex ratio of the brood they originally laid. To test this, I looked at the

feeding rates of parents in relation to the sex ratio of the original brood at experimental nests where the sex ratio was manipulated. I found no indication that parents are using the original sex ratio to set feeding rates. Male and female feeds/nestling/hour were not correlated with brood sex ratio (r_s = -0.05, p = 0.76; r_s = 0.003, p = 0.99), nor was the total feeds/nestling/hour (r_s = 0.01, p = 0.95) for the 32 manipulated nests in this study.

Discussion

These results indicate that parents do not adjust their feeding rates in response to manipulation of the sex ratio of the offspring in their nest. Given that there are behavioral and ecological differences between the sexes, many differences in cost have been hypothesized. For house sparrows, differences in dispersal tendencies and fledgling weights have been reported for other populations and I hypothesized that these could influence provisioning of sons versus daughters. In this population, however, female-biased dispersal and size dimorphism at fledging appear not to exist. Mean sex ratios at hatching for this population for 1994, 1995, and 1996 were 0.48, 0.48 and 0.45 respectively. The mean sex ratio at hatching did not vary significantly between years nor did it deviate from 0.5 (Voltura, chapter 1). Of the over 800 nestlings banded between 1994 and 1996, only 27 have been resighted near their natal area. Of those 27 individuals, 17 were females and 10 were males. Given that sampled nestling sex ratios do not differ from 0.5, this suggests that females are not more likely to disperse than males in this population. The results of the experiment also suggest that sons and daughters might not vary in weight at fledging, because the weights of male and female nestlings in control broods, measured a few days prior to fledging, did not differ.

However, there are still differences between sons and daughters in the variability of reproductive success. Extra-pair fertilizations have been found in this

population with 16 % of the nestlings being extra-pair offspring (R. Whitekiller, unpublished data) and some males are polygynous. If the observed levels of polygyny and EPFs do result in males having a greater variance in reproductive success, the results of this experiment suggest that these differences do not necessarily result in measurable differences in parental care during the nestling stage. A study on western bluebirds, *Sialia mexicana*, in which offspring sex ratios were manipulated late in the nestling period also found no adjustment of male and female feeding rates to nests with extreme sex ratio biases (Leonard et al. 1994). In western bluebirds, dispersal is female-biased and differential provisioning was predicted with parents expected to bias investment in favor of daughters. In that study, parents also did not preferentially feed either sons or daughters in mixed-sex nests.

Although sparrow parents are not adjusting feeding rates to broods with manipulated sex ratios, there were some differences within the control nests where sex ratio was not manipulated. Feeding rates by resident males tended to increase as the proportion of sons in the nest increased, although male and female nestlings had similar growth rates and were similar in weight at day 11 within control nests. The relationship between paternal feeding rates and brood sex ratio may be related to other findings on parental investment in house sparrows. While I did not find differential allocation to sons and daughters after hatching, some of my other work has shown that house sparrows may bias allocation to sons and daughters by producing biased sex ratios. Female house sparrows mated to large-badged males had broods with a more male-biased sex ratio than females mated to smaller-badged males (Voltura, chapter 2). Also, male badge size is positively correlated with the proportion of feeding visits made by the male (Voltura, chapter 3), suggesting that parents vary in their ability to invest in offspring and that larger-badged males may have more resources to invest in offspring. Overall, these results suggest that although sons and daughters may be equally costly during the nestling period, there are potential benefits to individual parents for

differential investment in sons and daughters. In some populations of house sparrows, male badge size is condition dependent (Veiga and Puerta 1996), while in others badge size has been found to have a heritable component (Møller 1989). Large-badged males may produce sons that inherit the large badge but they are also providing proportionately more feeding than small-badged males and thus may produce sons in better physical condition. If badge size is condition dependent in this population, then sons of large-badged males may develop larger badges at their first molt and thus increase their reproductive success in their first breeding season. These results agree with the predictions derived from the Trivers and Willard hypothesis: parents that are able to invest more in offspring are favored to bias allocation in favor of the sex with the higher variance in reproductive success. If house sparrow males have a higher variance in reproductive success than females, as suggested by the occurrence of EPFs and polygyny, then differential sex allocation by parents may be expressed in biased sex ratios rather than in differential parental care during the nestling period.

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Figure legends

Figure 1.   Mean feeding visits per nestling made by each parent for all three

treatment groups. Error bars are ± 2 SE, n=16.

Figure 2.   The mean proportion of feeds made by the father for nests in all

three treatment groups. Error bars are ± 2 SE, n=16.

Figure 3.   (a) Mean chick mass for each nest when nestlings were banded

before fledging. (b) Growth rates during the experiment

measured as mean mass gained per day. Error bars are ± 2 SE,

n=16.

Figure 4.   The sex ratio of each control brood correlated with: (a) the mean

feeds/nestling/hour for mothers, (b) the mean feeds/nesting/hour

for fathers, (c) the total number of feeds/nestling/hour and (d)

the proportion of total feeds/nestling/hour made by the father

(n=16).

Mean proportion of
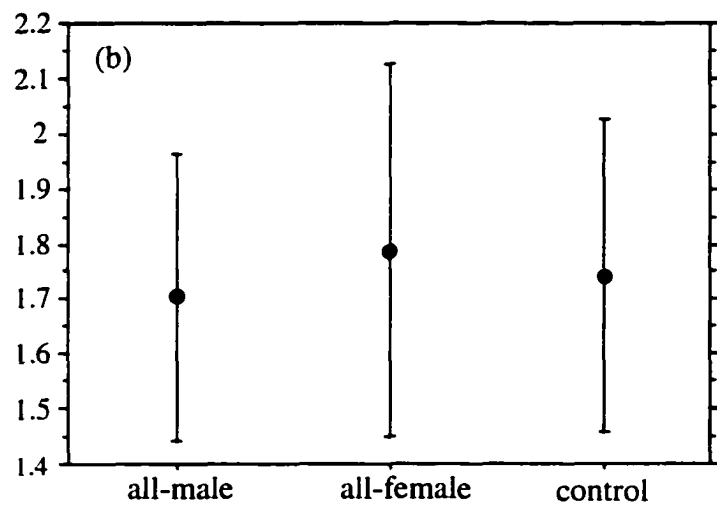feeds by father
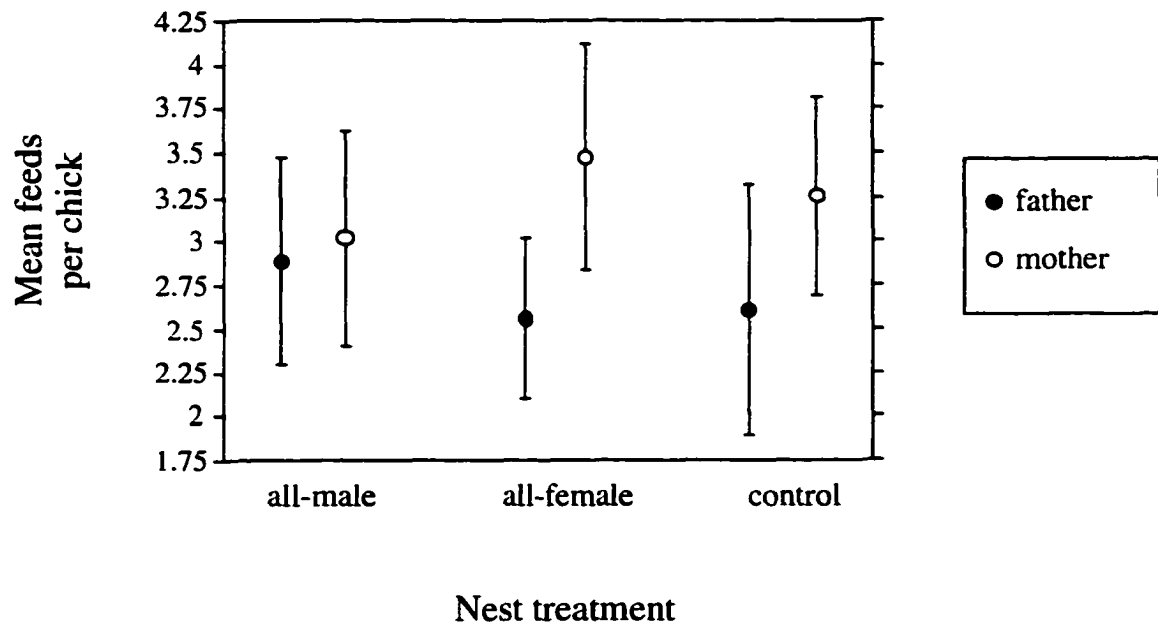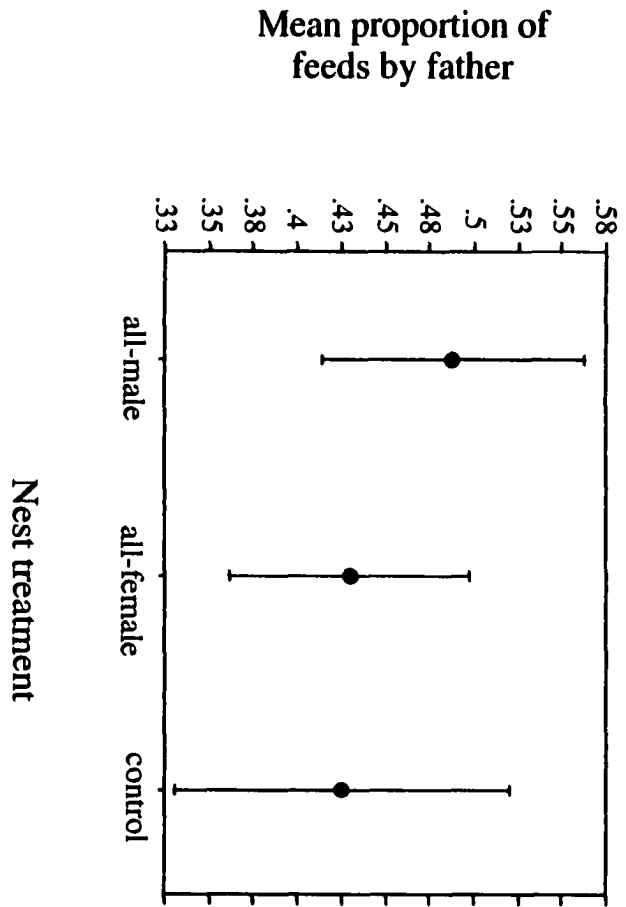
Nest treatment

Mean chick mass at day 11 (g)
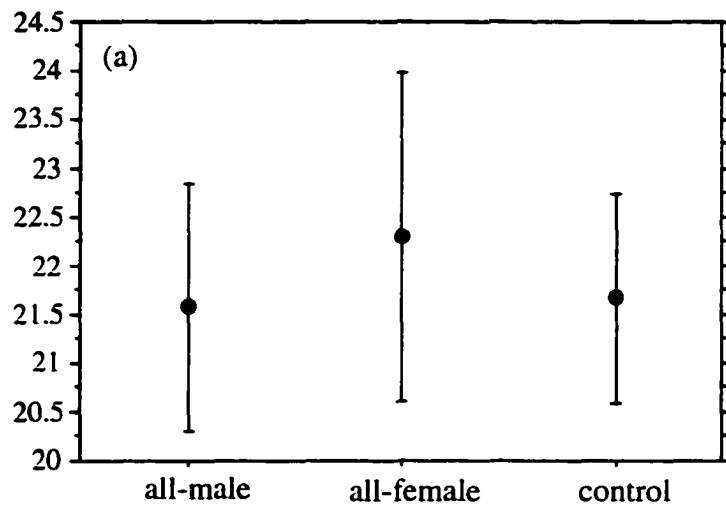
Mean mass gained per day (g)

Nest treatment

(a)

$r_s = -0.03, p=0.91$

Maternal feeds/nestling/hour

(b)

$r_s = 0.44, p=0.09$
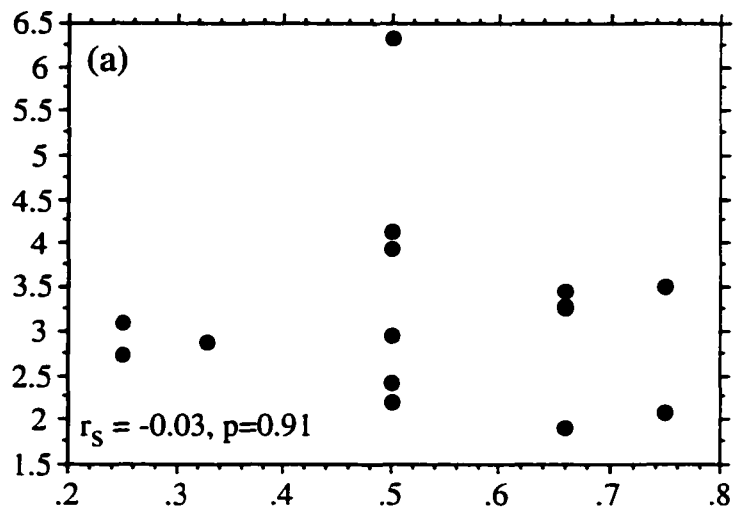
Paternal feeds/nestling/hour

Sex ratio

Total
feeds/nestling/hour

(c)

$r_s = 0.40, p=0.13$
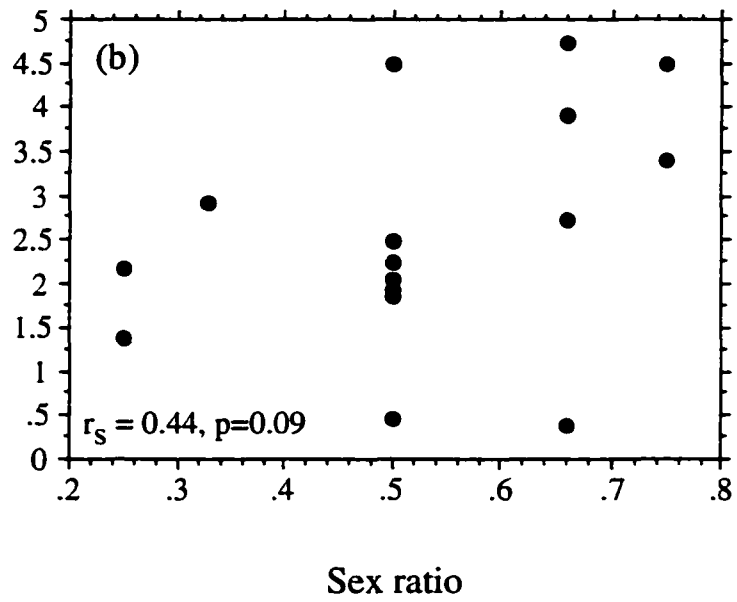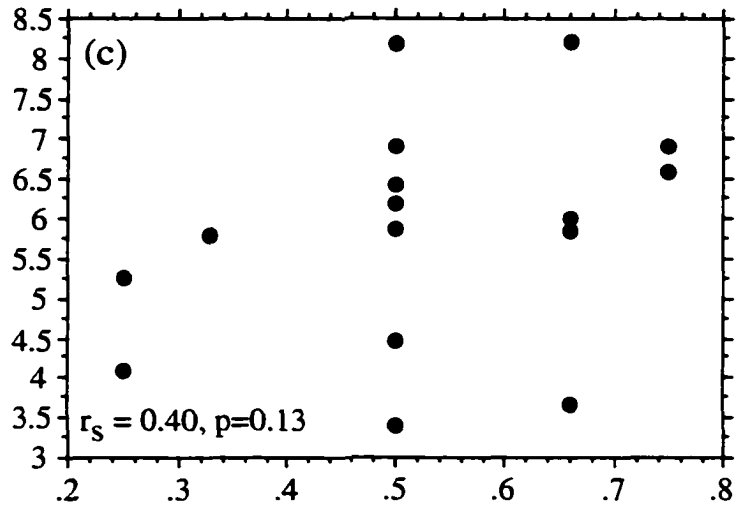
Proportion of total
feeds made by the male

(d)

$r_s = 0.31, p=0.23$
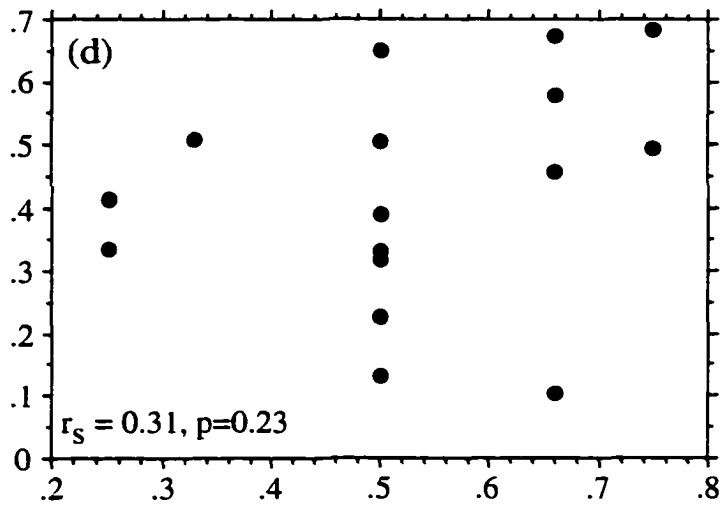
Sex ratio

Nestling Sex Ratios as Related to Hatch Order

in Cattle Egrets, *Bubulcus ibis*

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Abstract. Fisher's sex ratio theory states that equal investment in the two sexes is adaptive for sexually diploid taxa because sons and daughters have equal reproductive value. Trivers and Willard later hypothesized that sex-biased investment may sometimes be advantageous if one sex, usually male, has higher variance in reproductive success. Females in good condition should produce sons whereas females in poor condition should invest in daughters because these offspring should have lower variance in reproductive success. Trivers and Willard's argument can be extended to cases where variables other than parental condition influence offspring quality. In many avian systems factors such as hatch asynchrony and resulting hatch order may have effects on offspring survival that match or exceed parental condition. For cattle egret nestlings, asynchronous hatching, aggressive sibling competition, and facultative brood reduction result in favorable conditions for growth and survival of the first chick hatched and less favorable conditions for the last chick hatched within a three-chick brood. This leads to the prediction that the sex ratio of the first-hatched chicks in cattle egret broods should be biased towards males, the sex likely to have higher variance in reproductive success, and the sex ratio of the last-hatched chicks should be biased towards females. The sex of nestlings from three-chick broods of known hatch order was determined using flow cytometry. The overall sex ratio of nestlings was male-biased, due primarily to a strong male bias among the first hatched chicks. There was no corresponding female bias among the last-hatched chicks. Mothers may be manipulating the sex of the first chick to put sons in a position within the brood where they are most likely to survive.

Introduction

Fisher's (1958) theory of frequency-dependent selection predicted equal investment in male and female offspring such that if more parental investment were allocated towards one sex, the reproductive value of the opposite sex would increase in the population, automatically causing selection then to favor investment in the rarer sex. Trivers and Willard (1973) argued that sex biased investment patterns may be favored for individuals in polygynous mating systems. If males vary more in reproductive success than females, and if early investment is important for future reproductive prospects, then investment patterns may be biased in favor of sons for those parents that are in good condition. If daughters tend to have similar reproductive values, regardless of parental condition, but good sons have a much higher reproductive value than either average sons or all daughters, parents that can afford the cost of raising exceptional sons are favored to do so. In red deer, *Cervus elaphus*, male reproductive success varies more than female reproductive success. Consistent with Trivers and Willard's prediction, high-ranking females (i.e. those in better condition) produce more sons than daughters while females in poor condition produce more daughters than sons (Clutton-Brock et al. 1984). By contrast, in white-tailed deer, *Odocoileus virginianus* (Verme 1983), and several primate species (Clutton-Brock 1991) where reproductive success varies more among females than males, dominant females tend to have daughters and subordinate females tend to have sons.

By extension of the Trivers and Willard logic, if the competitive ability of progeny were influenced by factors other than parental condition, offspring sex ratio might be adaptively adjusted such that individuals in the relatively favorable positions might be of the limited sex; those in relatively less advantageous positions, of the limiting sex. In avian brood-reducing systems with asynchronous hatching, hatch

order is a reliable predictor of offspring growth rates and affects the probability of an offspring surviving the early nestling stage (reviewed in Mock and Parker 1997). Cattle egrets, *Bubulcus ibis*, exhibit facultative siblicide, and the asynchronous hatching of nestlings sets up a size hierarchy favoring the earlier hatched chicks. Parents facilitate asynchronous hatching by starting incubation before the clutch is completed and by manipulating the pattern of nest temperatures during incubation (Blaker 1969; Inoue 1981; Gieg in review). Mothers also vary the yolk steroid concentrations in eggs according to hatch order, with the a- and b-eggs containing double the levels of testosterone and related steroids than the c-egg (Schwabl et al. 1997). After hatching, the two senior siblings in three-chick cattle egret broods (the a- and b- chicks) consume about 80% of the food delivered to the nest (Ploger and Mock 1986) and have a one-third greater chance of surviving the first two weeks after hatching (Mock and Parker 1997). In times of low food availability, aggressive sibling competition typically results in very low feeding rates to the last-hatched chick, which is the most frequent victim of siblicide. Parents do not show overt preferences in feeding the eldest chicks, but food acquisition is dependent on position in the nest and access to the parent's beak during feeding, both of which are related to chick size and ability to exclude its rivals from the prime feeding positions (Fujioka 1985a, 1985b; Ploger and Mock 1986; Mock and Ploger 1987). Cattle egrets are size monomorphic and thus differences in size between male and female nestlings should not influence sex ratio variation.

In addition to the variation in condition across hatch order, there is also potential for variance in the reproductive value of male and female cattle egrets. As with many other avian species, cattle egrets are socially monogamous, but are well known to engage in frequent extra-pair copulations during the laying period (Blaker 1969; Lancaster 1970; Fujioka and Yamagishi 1981; McKilligan 1990; Krebs 1991). Recent DNA fingerprinting has revealed that the species is indeed genetically polygynous with

extra-pair fertilizations estimated at 15% (Gieg 1998). If male reproductive success does, in fact, vary more than female reproductive success, then any parental trait leading to the production of high-quality males could be favored.

To produce high-quality sons, cattle egret parents could manipulate the hatch order so as to ensure that the early-laid eggs (and the early-hatched chicks) tend to be male. If so, then in three-chick cattle egret nests, the sex ratio of the a-chick should be biased in favor of males. Similarly, one could argue that parents might bias the sex ratio of the last-hatched chicks towards females because a daughter of average or below-average quality is theoretically better than an average- or low-quality son.

In birds, it has long been assumed that a 1:1 primary sex ratio is the norm and that deviation from unity is constrained by random segregation of gametes during meiosis. However, with advances in molecular techniques, sex ratios of nestling birds are now easier to measure and recent evidence indicates that birds can and do vary the offspring sex ratio prior to hatching (Komdeur et al. 1997; reviews: Godfray and Werren 1996; Ellegren and Sheldon 1997). In this study, I tested the hypothesis that cattle egret parents bias allocation to the two sexes by manipulating the sex ratio of offspring across hatch order. I sexed cattle egret nestlings from 45 three-chick nests and tested the prediction that the sex ratio of the a- (first hatched) chicks would be biased towards males and the sex ratio of the c- (last-hatched) chicks would be biased towards females. I also examined brood sex ratios and hatch order for evidence of correlation with hatch date, a pattern that has been found for other birds (Dijkstra et al. 1990; Wiebe and Bortolotti 1992).

Methods

I sampled cattle egret broods at two nesting colonies in central Oklahoma, near the towns of Cement (1994) and Shawnee (1994 and 1995). Nests were marked during

nest building and then censused daily. The down of chicks was dye-marked on the day

of hatching to identify the a-, b- and c-chicks. For 10 nests, eggs were marked as they

were laid and chicks marked as they hatched. Laying order and hatching order were

identical for all ten nests. Only three-egg nests where all three chicks hatched were

used in the analyses. A small blood sample (< 50 μl) was collected in ACD

anticoagulant from each chick when it was less than 10 days old and stored at 4° C until

analysis. The sex of each chick was identified using flow cytometry, following the

protocol of Tiersch et al. (1989). Egret blood samples were combined with an internal

reference sample of catfish (*Ictalurus punctatus*) blood and stained with 0.5 ml of stain

containing 0.1% sodium citrate, 0.1% Triton-X100, 25 μl RNAse and 25 μg

propidium iodide. The stained cells were kept at room temperature and analyzed within

30 minutes.

The average DNA content per cell for each sample was calculated using the

median channel of the fluorescence intensity peaks according to the formula: pg egret

DNA = (B/C) * 2.0 pg, where B = bird median channel, C = catfish median channel

and 2.0 pg is the DNA content per cell for catfish (Tiersch et al. 1989). DNA content

for each unknown egret chick was compared to known-sex samples that were run

concurrently. These known-sex samples (n = 13 in 1994, n = 20 in 1995) were

collected from naturally dying cattle egret juveniles, the sexes of which were identified

by post-mortem dissection. The overall sex ratio across ranks was calculated, as were

separate sex ratios for the a-, b- and c-chicks. In cases where I was unable to identify

sex clearly using flow cytometry, neither that individual nor its nestmates were used in

the analyses. For the remaining sample of 45 three-chick broods, the sex ratios at each

rank in the nesting order and the overall sex ratio were tested with the Goodness of Fit

G-test, using Williams' adjustment (Sokal and Rohlf 1981). There was no difference

in the overall brood sex ratios across the two years of this study (Mann-Whitney

U=151.50, p=.23, n=45); therefore, the data for 1994 and 1995 were combined for the remainder of the analyses.

Results

The overall sex ratio was significantly male-biased (proportion males = 0.60; G_{adj} = 6.25, p=0.01, n=135) as was the sex ratio for the a-chicks (proportion males = 0.71; G_{adj} = 4.51, p=0.03, n=45) (Figure 1). Neither b-chicks (proportion males = 0.57; G_{adj} = 1.08, p=0.30, n=45) nor c-chicks (proportion males = 0.53; G_{adj} = 0.19, p=0.66, n=45) showed sex ratios deviating significantly from 0.5. To look for potential female biases in the last hatched chick I also looked at the sex ratio of the c-chicks for nests that had both sexes in the brood. For 30 nests that contained both male and female nestlings, the sex ratio of the c-chicks was 0.43, and was not different from a 50/50 sex ratio (G_{adj} = 0.263, p=0.61, n=30). Of the 15 single-sex nests, 4 were daughters and 11 were composed of only sons. Overall, there was significant heterogeneity among individual females (G_H = 66.30, p=0.02, n=45)

Hatching dates in 1995 ranged from 5 to 24 June and brood sex ratio (expressed as the proportion of males in a brood) was not associated with hatch date (r_s = -0.016, p=0.93, n=33). In 31 broods from 1995 where exact hatch dates for all 3 chicks were known, hatch interval (defined as the number of days between the hatching of the first and third chicks) was not correlated with sex ratio (r_s = 0.11, p=0.56, n=31). Furthermore, the sex of the chicks by rank was unassociated with both hatch date and hatch interval (Table 1.).

Discussion

The sex ratio results confirm that cattle egrets do bias the sex ratio of offspring, both for first-hatched chicks and for broods as a whole. This is consistent with the argument that parents could promote the production of highly successful sons by producing males in the hatching sequence where conditions are best for raising high quality offspring. However, there was no correspondent female bias for the c-chicks.

Adjustment of the sex ratio of the first- but not last-laid eggs may be a consequence of the possible mechanism by which avian sex ratio might be manipulated. Emlen (1997) suggested that parents are more likely to bias the sex ratio of the first-hatched chicks through resorption or abandonment of an egg belonging to the less advantageous sex. Such a strategy would be possible with the first egg of a clutch but probably not thereafter because discarding subsequent eggs would increase the laying interval, increase hatching asynchrony and presumably reduce fledging success. This may be the case with cattle egrets. The first-laid egg showed a significant male bias, but neither the second nor the third laid egg varied from a 50:50 sex ratio. In cattle egrets, increasing hatch asynchrony has been shown to increase the incidence of brood reduction (Mock and Ploger 1987). Females may be manipulating the sex of the first egg and then leaving the sex of subsequent eggs to chance to avoid various costs associated with increased hatching asynchrony. The bias of the overall nestling sex ratio in favor of males at hatching may simply be a consequence of the mechanism parents are using to bias the sex ratio of the a-chick.

Another suggested mechanism for sex ratio bias is the potentially different developmental rates of male and female follicles (Ankney 1982). Within a bird's ovary, follicles develop one at a time and if the development of male follicle is promoted by relatively low levels of reproductive hormones (either lutenizing hormone or follicle stimulating hormone), then the earliest maturing follicles would be skewed

towards males, with female follicles tending to mature later. This was one possible explanation for the patterns in snow geese (*Chen caerulescens*) where the first two eggs tend to produce males and the last two eggs tend to produce females. Because this is not interrupting random segregation of chromosomes at meiosis it would not affect the overall sex ratio, just the order of sexes produced. This explanation probably does not apply to cattle egrets because there is no compensating female skew of the later hatched chicks. This mechanism would be more plausible for cattle egrets if males were more likely to hatch before females in broods with both sexes, but using only mixed-sex broods, there is still not a significant skew of the c-chicks towards females. It would also be more plausible if an egg became Z- or W-bearing early in oocyte development. However, in chickens, the first meiotic division occurs just prior to ovulation (Schoenwolf 1995), and this would seem to preclude any variation in follicular growth rates based on chromosome composition of the developing oocyte.

With biases in the sex ratio at hatching it is becoming evident that birds can manipulate brood sex ratio prior to hatching and not merely through differential post-hatching mortality. There have been other studies that have shown sex ratio biases in relation to hatch order for sexually size dimorphic species (Table 2). In Australian peregrine falcons (*Falco peregrinus*), early hatched chicks are more likely to be females (the larger sex), perhaps because larger females can become more successful as breeders than small females (Olsen and Cockburn 1991). The opposite pattern is seen in Harris' hawks (*Parabuteo borealis*), where first hatched chicks tend to be males (the smaller sex) with the explanation that males are less expensive and that by placing the smaller sex first in the hatch order, the opportunity for unnecessary brood reduction is diminished (Bednarz and Hayden 1991). In European kestrels (*Falco tinnunculus*), the bias varies in relation to nesting date, with males hatching first in early nests and females hatching first in later nests (Dijkstra et al. 1990). In species where the size dimorphism is less pronounced, biases in relation to hatch order have also been

suggested. In one study of lesser snow geese (*Chen caerulescens*), the first two eggs tended to produce males and the last two eggs, females (Ankney 1982), but this pattern was not found in a different study population when several years of data were combined (Cooke and Harmsen 1983). In ring-billed gulls (*Larus delawarensis*), first eggs also produced more males (Ryder 1983), and c-eggs tended to produce females in two of five years, as well as in the pooled data for all years (Meathral and Ryder 1987). There was year-to-year variation in sex ratios and egg sequence and in the two years when females were in poor breeding condition, the overall sex ratios were female biased, indicating that females were biasing the sex ratio towards the less expensive sex. The year to year variation found in these studies suggests an adaptive response to environmental conditions. I found no difference in the sex ratios between the two years and the pattern of sex ratio and hatch order was approximately the same for both years.

First-hatched cattle egret chicks are more likely to survive the first two weeks of the nestling stage, but fledging success and long-term post-fledging survival rates are unknown in relation to hatch order for cattle egrets (Mock and Parker 1997). The effects of this bias within the hatch order on the sex ratio of adults in the breeding population are therefore also unknown. A long-term study on another brood-reducing species, the western gull (*Larus occidentalis*), showed that the two older siblings had higher survival during the nestling stage and for the first 9 months post-fledging than the last-hatched sibling, but those c-chicks that survived their first year were just as likely to breed as their older siblings (Spear and Nur 1994). If first-hatched chicks in cattle egret broods are more likely to survive to breeding age and are more likely to be male, this might skew the operational sex ratio and thus increase competition among males. The possibility that c-chicks may be able to overcome their slow start and survive to breeding age in brood-reducing species may prevent population sex ratios from becoming extremely skewed in favor of the sex that is overproduced first in the hatch order.

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Table 1. Results for the test for association between sex of the chicks at each rank in the hatch order with hatch date and hatch interval (Mann-Whitney U).

Rank in hatch order	hatch date (n=33)	hatch interval (n=31)
a-chicks	U=108, p=0.78	U=84.5, p=0.53
b-chicks	U=95.5, p=.015	U=106.5, p=0.62
c-chicks	U=118.5, p=0.53	U=118, p=0.93

Table 2. Other examples of sex ratio biases and hatch order in birds.

Species	Sex produced first	Size dimorphism	Hatch asynchrony	Reference
Perigrine falcon (*Falco peregrinus*)	female	female>>male	yes	Olsen and Cockburn 1991
Harris hawk (*Parabuteo borealis*)	male	female>>male	yes	Bednarz and Hayden 1990
European kestrel (*Falco tinnunculus*)	male (early season) female (late season)	female>>male	yes	Dijkstra et al. 1990
American kestrel (*Falco sparverius*)	male (early season) female (late season)	female>>male	yes	Wiebe and Bortolotti 1992
Lesser snow goose (*Chen caerulescens*)	male	none	no	Ankney 1983
Ring-billed gull (*Larus delawarensis*)	male	male>female	no	Ryder, 1983

Figure legends

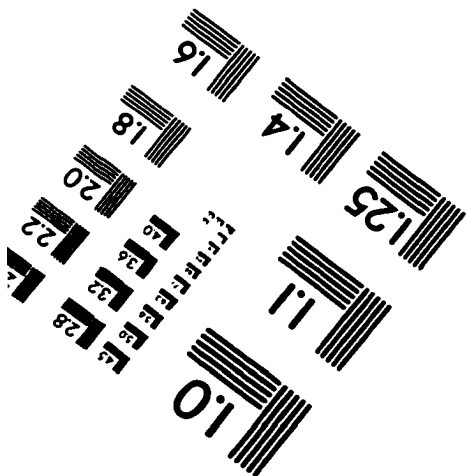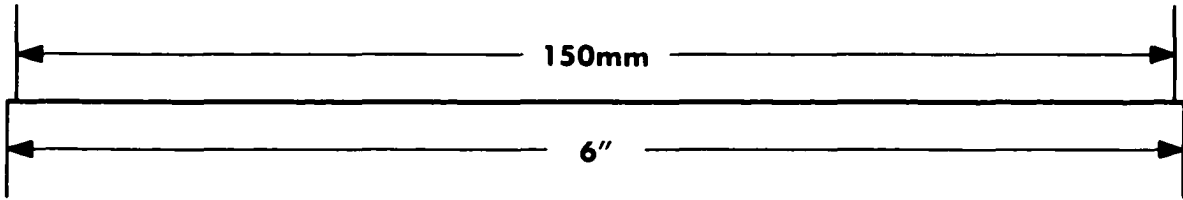Figure 1.   The sex ratios of all nestling ranks in 45 three-chick broods of cattle egret (* $p < 0.05$, William's G-test).
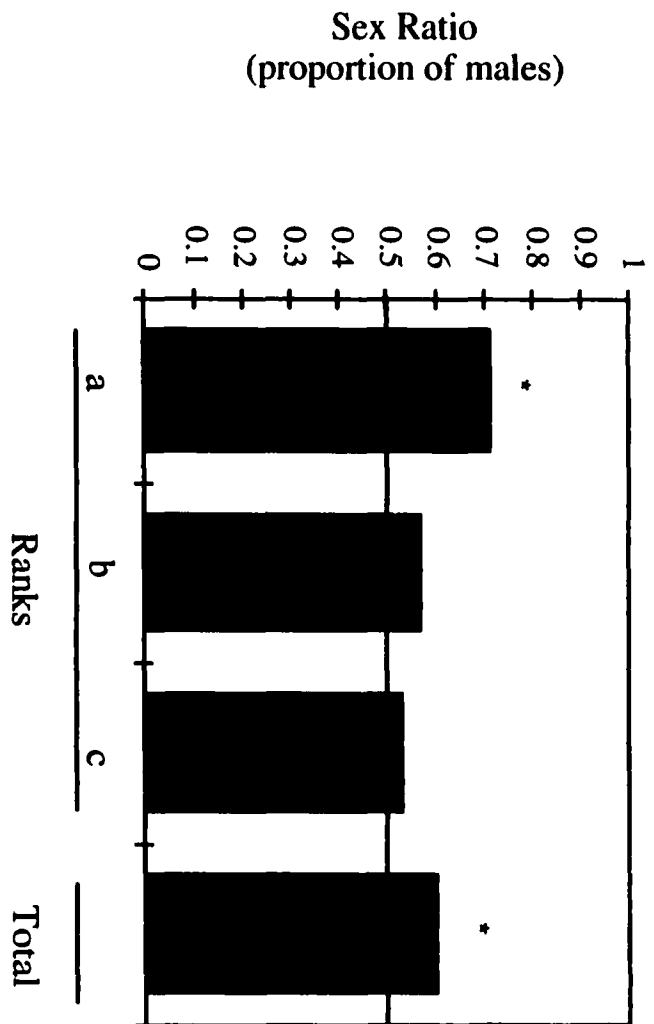
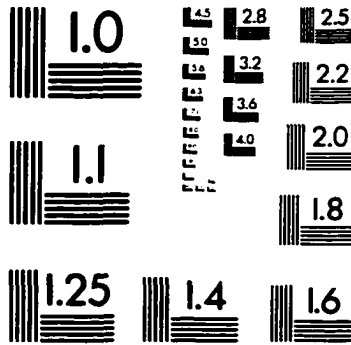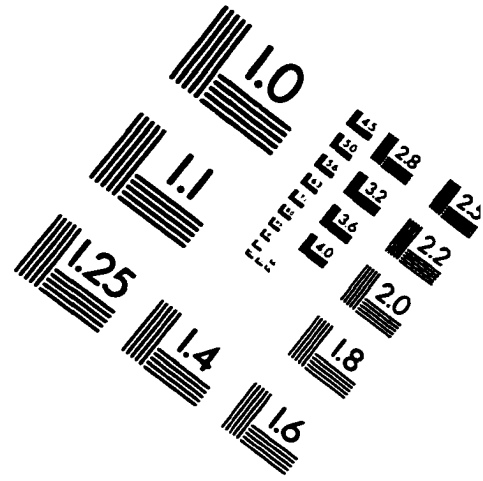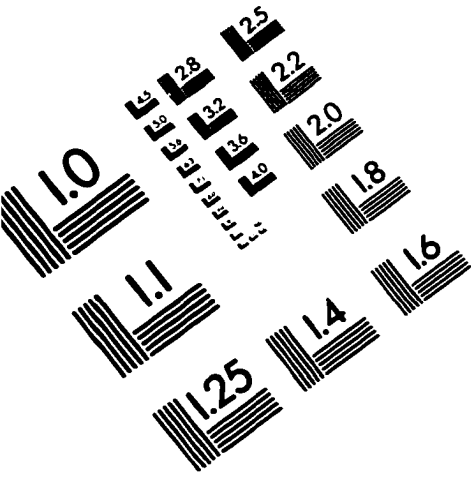# IMAGE EVALUATION
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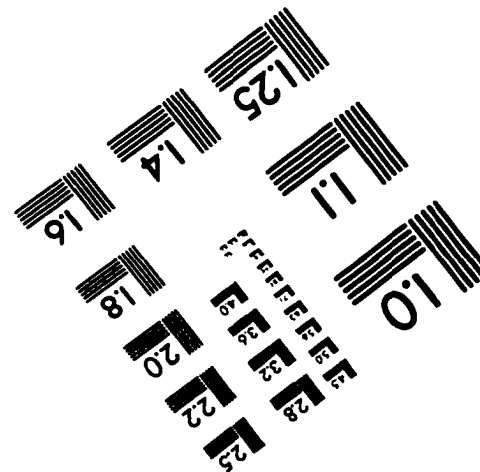
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