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SEXUAL SELECTION AND REPRODUCTIVE INVESTMENT
IN HOUSE WRENS (TROGLODYTES AEDON)

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SEXUAL SELECTION AND REPRODUCTIVE INVESTMENT IN HOUSE WRENS (TROGLODYTES AEDON)

By

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ABSTRACT

SEXUAL SELECTION AND REPRODUCTIVE INVESTMENT IN HOUSE WRENS (TROGLODYTES AEDON)

By

Natalie Suzanne Dubois

An individual's fitness depends on both the quality and quantity of offspring it is able to produce over its lifetime. For iteroparous species, the optimal allocation of investment to a particular breeding attempt should be mediated by its reproductive value relative to the costs to and benefits from future reproduction. Reproductive value might be influenced by mate or territory quality, environmental variables such as food abundance, or opportunities to secure additional matings. Within a breeding attempt, individuals can maximize reproductive success by choosing a high quality mate, investing more in offspring with high reproductive values, and taking advantage of additional mating opportunities if the benefits of doing so exceed costs. Individual trade-offs between investment in current and future reproduction may result in conflicts of interest between parents if, for example, a parent cannot simultaneously provision young and attract mates.

I investigated mate choice and reproductive investment decisions in the house wren (*Troglodytes aedon*), a facultatively polygynous cavity nester. In contrast to what is observed in a congener (winter wren, *T. troglodytes*), I found that the presence of empty (or 'cock') nests had no effect on mate choice or maternal investment in reproduction, but the availability of cavities within territories did affect male and female reproductive investment. Females mated to males with surplus nest boxes added to their territories laid larger clutches at early season nests and produced more males per clutch than females mated to males with single cavities in their territories. These results suggest that female house wrens might use cavity availability to assess male quality. Males with surplus nest boxes added to their territories sang more than males with single nest boxes, but this increased song rate had no effect on paternal provisioning efforts. Both males with surplus cavities and males with single cavities in their territories were less likely to provision at early season nests than at late season nests. Costs of provisioning at early season nests might be high for males, regardless of mate attraction opportunity, if males that provision at early season nests are less likely to rear second broods.

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final take-down. And it is with love that I thank my husband, Michael Hornish, who has always believed in me, even during those moments when I was not so sure myself.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Reproductive success depends on both the quantity and quality of offspring an individual is able to produce over his or her lifetime. For iteroparous species, investment in any given reproductive attempt should depend on the costs to future reproduction as well as the benefits of the current reproductive attempt (Stearns 1989, Hõrak 2003). An important factor affecting reproductive success is the quality of the mate or breeding situation. Individuals can maximize the value of a current reproductive attempt by choosing a high quality mate. The characters involved in mate choice may signal fitness benefits such as increased provisioning effort or increased viability of young (reviewed in Kirkpatrick and Ryan 1991, Kokko *et al.* 2003). Additionally, individuals can further increase the value of a reproductive attempt by investing more in reproduction when mate quality or the benefits provided by a mate are high (Burley 1986, reviewed by Sheldon 2000). Such trade-offs between current and future reproduction are a central component of life history theory (Stearns 1989). Of particular interest are situations characterized by sexual conflict in which the fitness interests of male and female parents diverge (Davies 1989).

Darwin (1871) identified sexual selection as the result of differences in reproductive success arising from competition over mates, either through intrasexual contests, generally males competing for access to females, or through intersexual mate choice. Sexual selection theory offered an evolutionary explanation for the prevalence of conspicuous secondary sexual traits such as the widowbird's tail (Andersson 1982), the elaborate constructions of bowerbirds (Borgia 1985), and the complex song repertoire of the great reed warbler (Catchpole 1980). Individuals might be able to use these signals to assess mate quality. For example, plumage coloration signals male capacity for parental care in house finches (Hill 1991), tail length signals offspring viability in barn swallows (Møller 1994), and song rate signals nest site quality in blackcaps (Hoi-Leitner *et al.* 1995).

Intersexual variance in reproductive success has traditionally been viewed as most apparent in polygamous systems, where some individuals mate with several individuals while others mate with few or none. Polygyny threshold models predicted that females settling with already-mated males are compensated for the costs of polygyny by acquiring a high quality breeding situation (*e.g.*, Orians 1969). Emlen and Oring (1977) proposed that the environmental potential for polygamy would depend on ecological factors such as the spatial distribution of resources. Polygyny would be favored when males could monopolize critical resources required by mates. Advances in our understanding of genetic versus social mating systems (*e.g.*, Westneat *et al.* 1990) have revealed female control over "resources" such as genetic parentage, and emphasize the importance of both sexes in shaping genetic and social breeding systems. For example, males might face trade-offs between mate-guarding and extra-pair copulations (Hasselquist and Bensch 1991), or, if females use male parental care at first brood nests in future reproductive investment decisions, males might use provisioning effort to guard parentage in subsequent broods (Freeman-Gallant 1996).

Preferred mates may sire more offspring through extra-pair copulations in monogamous as well as polygamous systems (sperm competition reviewed in Birkhead and Møller 1992). Even in the absence of sexual selection through extra-pair matings, differences in quality among mates could lead to variation in the quality and quantity of offspring produced. Preferred mates might produce more or higher quality offspring if they are able to attract higher quality mates (Parker 1983, Møller 1991), or if their mates invest more in reproduction (Burley 1986). For example, females mated to high quality males might increase provisioning rates (de Lope and Møller 1993), decrease rates of extra-pair copulation (Burley *et al.* 1994), lay larger eggs (Cunningham and Russell 2000), or increase levels of testosterone in eggs (Gil *et al.* 1999).

Conflicts of interest between the sexes over investment in mating opportunities and parental care may shape mating systems if the reproductive payoffs of monogamy and polygamy differ for males and females (Davies 1989). Gowaty (1996) suggested that facultative expression of male parental care might occur when the ecological opportunity for males to seek additional matings (either through extra-pair copulations or by attracting secondary females) depends on the ability of females to rear offspring alone within the environmental constraints of the given breeding situation. That is, males with mates of high social quality (able to rear young without assistance) would suffer less from a trade-off between mating effort and parental care. The relative importance of paternal care might depend on the ability of females to compensate for reduced care (Dunn and Hannon 1992, Sejberg *et al.* 2000), on environmental conditions (Bart and Tornes 1989, Kuitunen *et al.* 1996), or on the changing energetic demands of young (Johnson *et al.* 1992).

In my dissertation, I examine factors affecting reproductive investment decisions in house wrens (*Troglodytes aedon*). In this species, males are facultatively polygynous and are limited in their opportunity to attract additional females by the number of cavities (or nest sites) they can defend in their territory. Since males provide parental care by provisioning offspring, this sets up a potential conflict of interest between males and females in terms of investment in parental care. For males, investment in a given reproductive attempt may limit opportunities to attract additional mates, whereas for females, mate attraction effort by males (or the acquisition of a secondary female) may limit male contribution to parental care. Thus there are potential trade-offs in terms of the costs and benefits associated with allocation to current reproduction versus future reproduction for both males and females. Females make decisions regarding how much to invest in each reproductive attempt within the context of male quality and/or benefits received from the male. Males make similar decisions, but perhaps on a shorter time scale when opportunities to attract additional mates are high. Specifically, I have taken an experimental approach and addressed mate choice and parental investment decisions in several contexts. In Chapter 2, I examine whether empty nests, the result of egg removal from nests by house wrens, affect female mate choice and subsequent reproductive investment decisions by females. In Chapters 3 – 5, I focus on the effects of cavity availability on male and female investment in reproduction. I examine (a) whether the opportunity for males to acquire additional mates (*i.e.*, the presence of surplus cavities in a male's territory) affects a trade-off between mate attraction and paternal provisioning and (b) whether cavity availability affects mate choice and reproductive investment decisions by females.

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CHAPTER 2

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EMPTY NESTS DO NOT AFFECT FEMALE MATE CHOICE OR MATERNAL INVESTMENT IN HOUSE WRENS

with Thomas Getty

ABSTRACT

House Wrens (*Troglodytes aedon*) remove eggs from the nests of other birds, including conspecifics and heterospecifics and both cup and cavity nests. Egg removal by males before females arrive increases the number of empty nests in and around a male House Wren's territory, and females might use this trait in mate choice. We manipulated the presence of empty nests in House Wren territories prior to female settlement by adding artificial nests with or without plastic eggs. We used the timing of female settlement as an index of mate preference. Our manipulation had no effect on the timing of female settlement or on variables related to maternal investment such as clutch size, egg volume, or provisioning effort. Differential investment in offspring was based on the timing of a reproductive attempt, which was unrelated to the experimental manipulation.

Key words: egg removal, empty nests, House Wren, mate choice, nest destruction, parental investment, Troglodytes aedon.

INTRODUCTION

The propensity for egg removal and nest destruction by House Wrens (*Troglodytes aedon*) has been reported for over a century (Hill 1869, Creaser 1925, Kendeigh 1941, Kennedy and White 1996). Both sexes puncture and remove eggs from the nests of conspecifics and heterospecifics, but the behavior is suppressed in males after pairing and in females after the onset of egg laying (Belles-Isles and Picman 1986). Egg removal might accrue negligible costs, in which case the trait could be maintained as a vestige in the absence of current benefits. But there are several plausible hypotheses suggesting benefits of egg removal by House Wrens. House Wrens might destroy nests to reduce competition for resources such as nest cavities, food, or mates (Belles-Isles and Picman 1986) or to reduce predation risk by changing the ratio of active to inactive nests (Finch 1990).

There have been few critical tests of these hypotheses, due in part to the difficulties inherent in designing large-scale manipulations of the relevant factors. Several authors have documented cavity takeovers following nest destruction by male House Wrens (Quinn and Holroyd 1989, Pribil and Picman 1991), providing some support for a nest-cavity-limitation hypothesis, but these observations fail to explain the destruction of noncavity nests. Only the food competition and predation hypotheses can explain attack of both conspecific and heterospecific nests. Regardless of the ultimate function of egg removal, the major hypotheses focus on potential benefits accrued by a nesting pair in terms of increased reproductive success, and they also suggest direct benefits that choosy females might gain if mate choice were based on nest destruction (or its result, the presence of empty nests). Here, we take a different approach from previous studies, which have focused on the ecological function of egg removal, and ask instead whether females use empty nests as a mate-choice cue.

Female House Wrens might prefer males with empty nests in their territories if the presence of empty nests signals male or territory quality. We might expect females mated to preferred males to invest more in reproduction. This could occur if preferred males pair with higher quality females, or if females mated to preferred males have access to high quality territories. Additionally, females might allocate resources to reproduction in response to the perceived quality of their mates (Burley 1986). Examples of differential allocation by females mated to high quality males include increased provisioning effort (de Lope and Møller 1993, but see Witte 1995), increased clutch size (Petrie and Williams 1993), and larger eggs (Cunningham and Russell 2000).

Male House Wrens control the number of empty nests in their territories not only by egg removal but also by placing sticks in unoccupied cavities (Kendeigh 1941). The evidence that female House Wrens attend to nest site characteristics is mixed. Secondary females in a polygynous population of House Wrens preferred males with front-entrance nest boxes over males with roof-entrance boxes (Johnson and Searcy 1993), but there is no evidence that the size of a male's nest influences female choice or reproductive success (Alworth 1996). Neither of these studies addressed the effect of male manipulation of multiple nest sites on female mate choice. Here we present the results of a nest-site manipulation study in a primarily monogamous, double-brooded population of House Wrens. We test the hypothesis that female House Wrens prefer males with empty nests over males with egg-containing nests in their territories and examine subsequent patterns of reproductive investment.

METHODS

House Wrens are small (10-12 g), migratory, secondary cavity nesters (Johnson 1998). Male House Wrens in our population arrive in late April, and the first females arrive several days to a week later. Upon arrival, males establish territories and begin constructing nests by placing a few to hundreds of small sticks in available cavities. Females inspect cavities before selecting a nest site, and once the nest cup is completed, line the nest with soft material (Kendeigh 1941). House Wrens in our population frequently double-brood, with early season clutches generally initiated in May and late season clutches initiated mid June through July. We considered nest lining the date of nest initiation.

We conducted this study April through August of 1999 and 2000 at the Kellogg Biological Station's Lux Arbor Reserve (42°29'N, 85°28'W) in Barry County, Michigan. Lux Arbor Reserve is a 529-ha site consisting of fragmented habitats including hardwood and softwood forests, open fields, wetlands, and agricultural areas. We installed nest boxes in clusters (hereafter referred to as sites) composed of four nest boxes spaced 5 m apart. Boxes were placed along forested edges wherever possible. We did not control the orientation of the box entrance, but this characteristic appears to have little effect on nest site preferences in House Wrens (Lumsden 1986). In March 1999, 36 sites were established approximately 100 m apart. Four sites were added in April 2000. All nest boxes were installed on metal poles and placed approximately 1.2 m above the ground. To minimize losses due to predation, the poles were greased at the beginning of the breeding season in 2000, and a 0.75-m conical predator guard was installed on the poles of all active nests.

Experimental manipulation

We checked nest boxes at least every other day during male and female settlement periods and at least every 4 days during the nesting cycle (usually more often). We set up the experiment within 1-3 days of finding a nest box with at least 20 sticks in it and always prior to female nest lining. We designated the nest box with the most sticks in it as the primary nest. This nest was not manipulated. Experimental manipulations were set up in the remaining (dummy) nest boxes. In one half of sites, empty artificial nests (made of dried grass) were placed in dummy nest boxes to simulate nests from which eggs had been removed. In the remainder of sites, artificial nests containing a clutch of artificial plastic eggs were placed in dummy nest boxes to simulate the presence of active nests. Artificial nests were placed in nest boxes at a height approximating that of a fully lined House Wren nest (approximately 3 cm below the entrance hole). Dried Spanish moss (*Tillandsia usneoides*) was used to disguise the materials supporting the artificial nest in the nest box. The artificial eggs were slightly smaller than wren eggs (15 mm in length) and cream in color with brown speckling, but House Wrens are not choosy about the characteristics of the eggs they remove (Belles-Isles and Picman 1986). We wired artificial eggs into nests to prevent removal by House Wrens. We removed sticks placed over the artificial nests by House Wrens, except in cases where lining material was also present.

We paired territories by male settlement date (which we assumed was the date we first observed sticks in a nest box at a site) and randomly assigned treatments within those pairs. In 1999, only a subset of early season nests was manipulated (*n* = 19). In 2000, both early (*n* = 36) and late season nests (*n* = 18) were manipulated. Early nests were defined as nests lined before 11 June 2000 (the median lining date). Nests lined after that date were classified as late broods regardless of whether one or both members of a pair were known to have fledged an earlier brood. Dummy nest boxes were cleaned out several days before nestlings fledged at the primary box in order to allow males to begin building late season nests. Primary nest boxes were cleaned out after nestlings fledged.

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We randomly reassigned treatments to males building late season nests. Nests initiated after a successful first brood were almost always built in former dummy boxes. We included each House Wren pair only once in analysis by excluding late season nests of double-brooded pairs from the data set (except for provisioning data from one pair for which we only recorded data during the second brood). Late season nests of novel pairs (either as a result of mate switching or the appearance of new breeders to our study site) were included in our data set.

Behavior and reproductive success

In order to assess whether the experimental treatment affected male behavior prior to female settlement, we conducted 20-min focal observations 1-2 days after installation of the experimental treatments on a subset of males ($n = 23$) during the early nesting period in 2000. We recorded at 15-sec intervals whether a male was singing, and whether a male was in or on a primary or dummy nest box. We initiated an observation only when we located a male on the territory either by sight or sound. We calculated male time budgets from these data.

The number of days a male remained unmated was calculated as the lag between the date we first observed sticks in a box at a site and the date of female nest lining (which we used as an index of pairing date). In cases where we missed the first sign of lining and found a lined nest containing an egg on a subsequent visit, we used the day between our nest checks as the nest-lining date.

We used clutch size, egg volume, nestling mass and number, and parental provisioning rates as our measures of reproductive investment. We measured egg length and breadth to the nearest 0.1 mm using dial calipers and estimated egg volume (in mL) using the formula, *length* × *breadth*^{2} × 0.000491 (formula in Johnson 1998). In 2000, nestlings were counted and weighed with a spring scale on brood day 12 (brood day 0 = hatch). Nests that failed partway through the nesting period were included in analyses based on data collected before the failure.

We observed provisioning trips for 30-min periods in the morning (between 06:00 and 11:00 EST) on brood days 4, 8, and 12. Any parental visit to the entrance of the nest box was considered a provisioning trip, regardless of whether we actually observed food. We captured adults prior to provisioning observations using a mist net placed in the territory or a box trap, and marked each adult with an aluminum U.S. Fish and Wildlife Service leg band and a unique combination of three color bands. Trapping and banding was conducted under the appropriate federal, state, and university permits. Provisioning observations were conducted during fair weather using 10x binoculars mounted on a tripod, taking care to minimize disturbance. We excluded from analysis any observations during which neither parent resumed provisioning.

Statistical analyses

Statistical analyses were performed using SAS v8.1 (SAS Institute Inc. 1999). We used Wilcoxon rank sum tests to compare premating male time budgets. All other analyses were performed using parametric tests, transforming data when necessary to improve normality. Settlement data were log transformed and provisioning data (counts) were square-root transformed. We included only complete clutches in our analysis of clutch size and egg volume. We excluded renests from our analyses for two reasons: (1) females might invest differently in egg number and size after a nest failure, and (2) renests

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renests were often built in dummy boxes over the artificial nests, thus disrupting the experimental manipulation.

The effects of the experimental manipulation on settlement and reproductive success data were analyzed using ANOVAs with season as a blocking factor (3 levels: 1999 early brood, 2000 early brood, 2000 late brood, except for nestling mass models for which we had no 1999 measurements). Provisioning rates were analyzed using the MIXED procedure in SAS (repeated measures with a compound symmetry covariance structure). In instances where we report only data on provisioning rates per nestling, we also conducted these analyses per nest and results from both analyses were essentially the same. In addition to treatment, the following factors were included in provisioning rate models: season (2 levels: 2000 early brood, 2000 late brood), observation day (repeated measure, 3 levels: brood days 4, 8, and 12), and all interactions. We used the SLICE option in SAS to test simple effects of treatment within each level of brood for significant treatment × season interactions.

Estimates for power were based on Cohen (1988) and calculated using PASS (Hintze 2001). In cases where we failed to demonstrate a significant nest treatment effect we calculated 95% confidence intervals on the effect size (Steidl and Thomas 2001) and also back-transformed the upper limit into the measured variable where appropriate (Table 2.1). For comparison, means for transformed data are presented in untransformed units with 95% confidence limits (based on analysis of the transformed data). All other values are presented as means ± SE.

RESULTS

Male response to artificial nest supplementation

The addition of empty nests or egg-containing nests to male House Wren territories had minor effects on male behavioral time budgets prior to mating. We did not observe significant differences in singing activity (empty vs. egg-containing nests: 54.8 ± 2.1, vs. 45.7 ± 2.3 observations out of a possible 81; $S = 113.0$, $P > 0.2$) nor in time spent in or on the primary box (0.6 ± 0.2 vs. 3.3 ± 0.5 observations; $S = 89.0$, $P > 0.5$), although the power to detect a medium effect size was low for both tests (power = 0.2). Males with empty nests added to their territories spent significantly more time in or on dummy boxes than males with egg-containing nests added to their territories (3.3 ± 0.5 vs. 0.3 ± 0.04 of 81 observations respectively; $S = 124.5$, $P < 0.03$).

Settlement patterns

Of first-brood pairs included in the experiment, more than 50% of males had established territories by the time the first nest was lined in the study population (1999: 13 of 19 males, 2000: 21 of 36 males). Therefore, we assumed that females could choose among males. The experimental manipulation did not affect the number of days a male remained unmated (back-transformed means [95% CI] for empty vs. egg-containing nests: 7.4 [5.7-9.6] vs. 9.4 [7.5-11.7] days; $F_{1,63} = 1.9$, $P > 0.1$).

Reproductive investment

The experimental manipulation had no effect on our measures of reproductive success: clutch size (empty vs. egg-containing nests: 6.2 ± 0.2 vs. 6.2 ± 0.1 eggs; $F_{1,55} < 0.01$, $P > 0.9$), average egg volume per nest (empty nests = egg-containing nests = 1.3 ± 0.02 mL; $F_{1,50} = 0.1$, $P > 0.7$), number of nestlings in the nest on brood day 12 (empty vs. egg-containing nests: 5.6 ± 0.3 vs. 5.5 ± 0.2 nestlings; $F_{1,36} = 0.06$, $P > 0.8$), or average nestling mass per nest on brood day 12 (empty vs. egg-containing nests: 10.0 ± 0.2 vs. 10.2 ± 0.1 g; $F_{1,28} = 0.5$, $P > 0.5$).

Combined provisioning rates. There was no significant treatment effect on the total number of provisioning visits per 30 min made by both parents, but there was a significant treatment × season interaction (treatment, $F_{1,31} = 0.2$, $P > 0.6$, treatment × season, $F_{1,31} = 6.7$, $P < 0.02$). Comparisons of treatments within a brood indicate that pairs with empty nests added to their territories made more provisioning visits than pairs with egg-containing nests added to their territories during early broods ($P < 0.01$) but not late broods ($P > 0.2$, Figure 2.1). Provisioning rates per nestling showed similar patterns (treatment, $F_{1,32} = 0.01$, $P > 0.9$, treatment × season, $F_{1,32} = 4.0$, $P < 0.06$).

Individual provisioning rates. There was no significant treatment effect on provisioning rates per nestling by males, but there was a significant treatment × season interaction as well as a strong season effect on male provisioning rates per nestling (Figure 2.2a; treatment, $F_{1,32} = 0.1$, $P > 0.7$, season, $F_{1,32} = 10.5$, $P < 0.01$, treatment × season, $F_{1,32} = 4.5$, $P < 0.05$). The experimental manipulation had no effect on provisioning rates per nestling by females, but there were significant seasonal effects on female provisioning (Figure 2.2b; treatment, $F_{1,33} = 0.1$, $P > 0.7$, season, $F_{1,33} = 4.4$, $P < 0.05$, treatment × season, $F_{1,33} = 0.9$, $P > 0.3$). Seasonal differences in female provisioning rates were opposite those observed for males (Figure 2.2).

DISCUSSION

We found no evidence that the addition of empty or egg-containing nests to male House Wren territories affected female mate choice. Male Winter Wrens (*Troglodytes*

troglodytes) build empty nests, which females use as a mate choice cue (Evans and Burn 1996). However, multiple nest building in Marsh Wrens (*Cistothorus palustris*) does not affect female mate choice but may affect reproductive success (Leonard and Picman 1987). Depredated Marsh Wren nests had fewer empty nests around them than successful nests (Leonard and Picman 1987). Before we installed predator guards, predators in a section of our study area destroyed nests regardless of their contents or the contents of surrounding dummy nests (some baited with quail eggs). This pattern suggests that dummy nests in our study area have no effect on nest predation from the most common predator, raccoons (*Procyon lotor*).

We found no differences in our measures of reproductive investment for female House Wrens mated to males with egg-containing nests in their territories and those mated to males with empty nests. Although the power of these tests was relatively low, we would have been able to detect an effect size roughly half the observed decrease in clutch size between broods. In addition, we observed extremely small effect sizes for our measures of reproductive success. Combined with the lack of a consistent pattern in the direction of the treatment effect on these variables, we consider it unlikely that the presence of empty nests has a biologically significant effect on maternal investment. We found a significant treatment × season interaction on combined parental provisioning rates to early nests. In early broods, pairs with egg-containing nests added to their territories made fewer nestling provisioning visits than pairs with empty nests added to their territories. Even though individual parental provisioning rates tended to show the same pattern, the interaction was significant only for males, and comparisons of treatments within a brood were not significant. Time budgets from the premating period provide no evidence that males with egg-containing nests added to their territories wasted a lot of time trying to remove eggs (in fact, they spent less time in dummy boxes than males with empty nests added to their territories). Although it seems unlikely, we cannot rule out the possibility that these males (or pairs) perceived increased competition or predation risks as a result of the manipulation and allocated time to other activities such as territorial defense at the expense of provisioning effort. In any case, the differences in provisioning rates observed among treatments had no effect on nestling mass on brood day 12.

We did observe seasonal differences in parental investment and reproductive success. We documented increased provisioning rates by male House Wrens late in the season when the potential to secure additional mates was likely reduced. These results are consistent with the idea that male House Wrens demonstrate adjust their investment in reproduction based on the possibility of attracting additional mates. We are currently testing this hypothesis.

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Table 2.1. Observed effect sizes (± 95% CI) and power to detect a medium effect size (f = 0.25, Cohen 1988) for dependent variables for which we failed to find a significant treatment effect or treatment × season interaction between house wrens with empty nests added to their territories and house wrens with egg-containing nests added to their territories. The upper limit of the observed effect size has been back-transformed into original units where appropriate.

Variable	df	*F*	*P*	Effect size (± 95% CI)	Upper limit	Power
Days male unmated	1, 63	1.9	0.1	0.10 ± 0.14[a]	1.7	0.53
Clutch size (eggs)	1, 55	<0.01	0.9	0.01 ± 0.40	--	0.48
Egg volume (mL)	1, 50	0.1	0.7	0.01 ± 0.05	--	0.45
Nestling number	1, 36	0.1	0.8	0.10 ± 0.59	--	0.35
Average nestling mass (g)	1, 28	0.5	0.5	0.17 ± 0.40	--	0.28
Female provisioning rate (visits per nestling per 30 min)	1, 33	0.1	0.7	0.04 ± 0.23[b]	0.07	[c]

[a] Log transformed

[b] Square-root transformed

[c] Feeding rates were analyzed using proc mixed in SAS which does not calculate sums of squares.

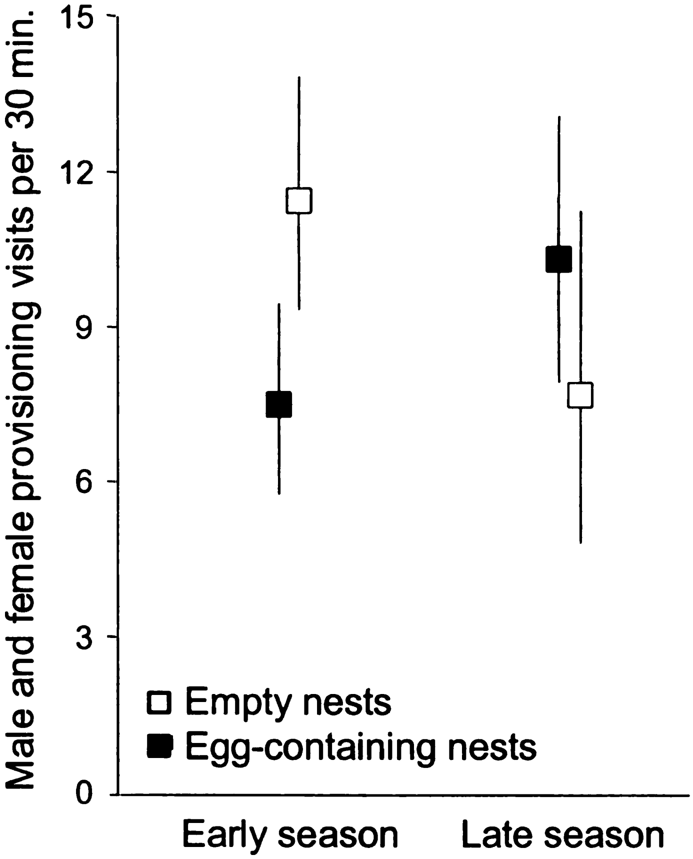

Figure 2.1. Average provisioning rates (both parents combined) at early and late season nests for house wren pairs with empty nests (unfilled squares) and pairs with egg-containing nests (solid squares) added to their territories. The treatment × season interaction was significant ($P < 0.02$). During the early season, pairs with empty nests added to their territories provisioned at higher rates than pairs with egg-containing nests added to their territories ($P < 0.01$). There were no differences in provisioning rates for late season nests. Back-transformed means ± 95% confidence intervals are shown.

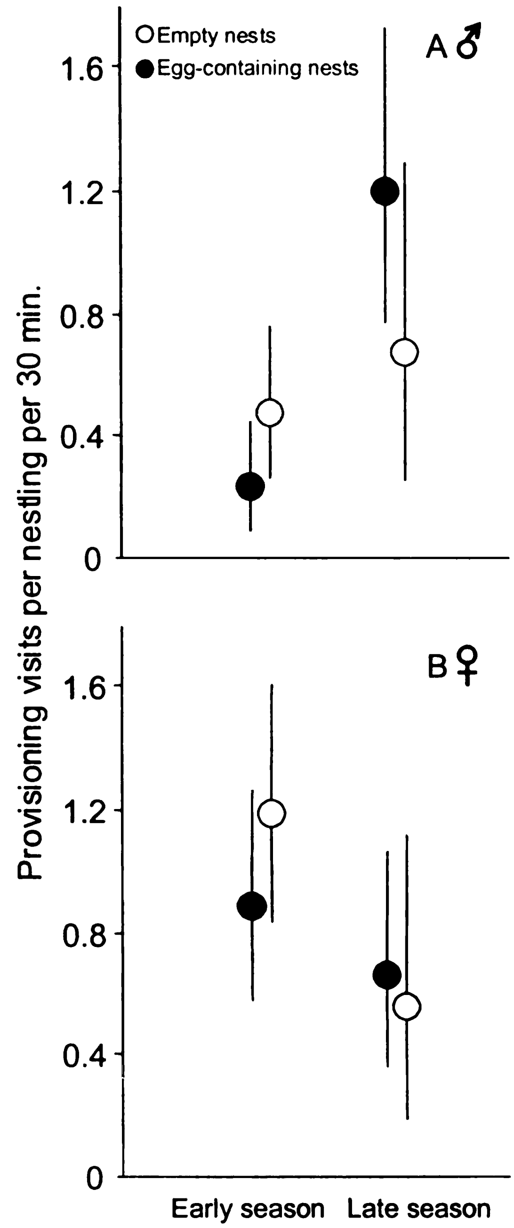

Figure 2.2. Average (a) male and (b) female provisioning rates per nestling at early and late season nests for house wren pairs with empty nests (unfilled circles) and pairs with egg-containing nests (solid circles) added to their territories. The treatment × season interaction was significant ($P < 0.05$) for males but not for females. For males, pairwise comparisons of treatments within a brood were not significantly different. Seasonal means were significantly different for males ($P < 0.01$) and females ($P < 0.05$). Back-transformed means ± 95% confidence intervals are shown.

CHAPTER 3

DOES CAVITY AVAILABILITY AFFECT TRADE-OFFS BETWEEN CURRENT AND FUTURE REPRODUCTION IN MALE HOUSE WRENS?

ABSTRACT

Parental investment in a given reproductive effort is generally constrained by trade-off costs to future reproduction. For polygynous and multi-brooded species these future costs can be almost immediate if investment in a current reproductive effort decreases the opportunity to attract additional mates. As a result, increased opportunity to attract additional mates might shift the balance towards less investment in early broods. I examined this trade-off in a polygynous, double-brooded passerine, the house wren (*Troglodytes aedon*), by experimentally manipulating opportunities for males to attract additional mates. Since cavity availability limits a male's opportunity to acquire additional mates, I manipulated male mate attraction opportunity by controlling male access to additional cavities. Newly settled territorial males with one nest box were randomly assigned zero or two additional cavities. I recorded male song as an indicator of mate attraction effort and monitored paternal provisioning as a measure of parental investment. Although males with surplus cavities in their territories sang more, paternal provisioning was not affected by cavity availability. Paternal provisioning rates were observed during the middle to late nestling periods, and reproductive trade-offs might have occurred earlier in the nestling period when females were brooding young. Regardless of treatment, males provisioned less at early season nests than at late season nests. The costs of investing in parental care at early season nests might be high,

regardless of mate attraction opportunity, if paternal care towards the end of the nestling period affects the probability of rearing a second brood.

INTRODUCTION

For iteroparous species, parental investment in a given reproductive attempt is generally constrained by trade-off costs to future reproduction and survival (Stearns 1989). These trade-offs can be difficult to study using correlational approaches because uncontrolled variables among individuals, such as resources, may result in positive relationships between traits across individuals, thereby masking negative trade-offs within individuals (van Noordwijk and de Jong 1986). For example, an individual in good condition might allocate more to reproduction and also have higher survival than an individual in poor condition, even though each individual faces a negative trade-off between investment in reproduction and survival. Experimental manipulations of phenotype (Sinervo and Basolo 1996, also "phenotypic engineering" Ketterson *et al.* 1996) can randomize phenotypes across individuals, thus breaking up naturally co-occurring traits and exposing underlying trade-offs.

Phenotypic manipulation might be achieved by (a) direct manipulation of a trait, (b) manipulation of the environment in order to produce a change in a trait, or (c) manipulation of a physiological mechanism that links multiple traits (Sinervo and Basolo 1996). To examine trade-offs between current and future reproduction, investment in current reproduction might be manipulated by increasing clutch size (*i.e.*, an environmental manipulation affecting parental investment in current reproduction). For example, female collared flycatchers rearing experimentally enlarged clutches produce fewer offspring in subsequent reproductive attempts, demonstrating a trade-off between current and future reproduction (Gustafsson and Pärt 1990). Another possibility is to manipulate a proximate physiological mechanism, such as a hormone, that underlies the trade-off. For instance, testosterone affects trade-offs between territorial defense, a component of mate attraction, and survival in male mountain spiny lizards (*Sceloparus jarrovi*, Marler and Moore 1988). In dark-eyed juncos (*Junco hyemalis*), males implanted with testosterone decrease provisioning effort (Ketterson *et al.* 1992) but produce more extra-pair young than control males (Raouf *et al.* 1997).

In the case of polygamous or multi-brooded species, trade-off costs to future reproduction can be almost immediate if investment in a current reproductive attempt (*e.g.*, parental care) decreases the opportunity to attract additional mates. Under this trade-off, we would expect increased opportunities for additional breeding attempts to result in decreased investment in current reproduction. Male two-spotted gobies (*Gobiusculus flavescens*) reduce levels of parental care when fertile females are present (Bjelvenmark and Forgren 2003), and fairy martins (*Hirundo ariel*) decrease parental care when opportunities for extra-pair matings are high (Magrath and Elgar 1997). When opportunities to attract additional mates are limited by nest sites, the availability of nest sites might affect trade-offs between investment in parental care and mate attraction. This trade-off has been observed in European starlings (*Sturnis vulgaris*), where already-mated males with an additional nest site added to their territory traded-off investment in parental care for increased mate attraction effort (Smith 1995).

Most studies that have manipulated trade-offs between investment in current and future reproduction have focused primarily on investment decisions of one parent and disregarded potential responses by the mate. For species with bi-parental care, the optimal level of investment by one parent may depend on the level of investment of its mate (Chase 1980, Winkler 1987, Lazarus 1990). If individuals are able to assess the liklihood that a potential mate will invest in parental care, this information might be used in mate choice and reproductive investment decisions. For example, if nest site availability mediates a trade-off between investment in mate attraction and parental care for males, then females might be able to use cavity availability to assess potential mates.

I investigated reproductive investment in a secondary cavity nester, the house wren (*Troglodytes aedon*). Song is an important component of mate attraction in house wrens (Johnson and Searcy 1996). Unmated males produce 'high volume spontaneous song' that ceases immediately after pairing but resumes during incubation, presumably to attract additional mates (Johnson and Kermott 1991). Previous studies have found that polygynous males have greater reproductive success than monogamous males (Soukup and Thompson 1997), and that paternal care is important to female reproductive success (Johnson *et al.* 1992, Johnson *et al.* 1993). These potentially competing interests set up a possible trade-off between investment in current and future reproduction if males cannot simultaneously invest in mate attraction and care for young. Male opportunities to attract additional mates are limited by the number of defendable cavities in their territory, and therefore, females might be able to use cavity availability in male territories to assess aspects of male quality such as parental investment.

In earlier experiments, I documented a significant difference in paternal provisioning effort between early and late season nests in house wrens (Chapter 2). Males provision late season broods at higher rates than early season broods. This increase in provisioning effort coincides with reduced mating opportunity late in the breeding season and suggests that male house wrens might increase paternal effort when opportunities to attract additional mates are low. I experimentally tested this hypothesis by manipulating cavity availability in male house wren territories. Since cavity availability limits a male's opportunity to acquire additional mates, I manipulated male mate attraction opportunity by controlling male access to additional cavities. I evaluated the effect of increased mate attraction opportunity on parental investment by both males and females. In this chapter, I examine whether cavity availability affects a trade-off between male investment in mate attraction and parental care.

METHODS

I conducted this study April through August of 2001 and 2002 at the Kellogg Biological Station's Lux Arbor Reserve (42°29'N, 85°28'W) in Barry County, Michigan. Lux Arbor Reserve is a 529-ha site consisting of fragmented habitats including hardwood and softwood forests, open fields, wetlands, and agricultural areas. Cedar nest boxes were installed in clusters (hereafter referred to as sites) composed of three boxes spaced 5-10 meters apart. All nest boxes were installed on metal poles and placed approximately 1.2 m above the ground. To minimize losses due to predation, a 0.75-m conical predator guard was installed on the poles of all active nests. Fifty-three sites were established, and each site was separated by a minimum distance of approximately 100m, which limited virtually all males to a single site per territory.

Male house wrens arrive at the study area in mid to late April and immediately begin establishing territories. Males begin placing sticks in available cavities shortly after arrival, and the first females arrive several days later. Once paired, females may help build the stick platform of the nest (Alworth and Scheiber 2000) and then line the nest cup with soft material (Kendeigh 1952, McCabe 1965). On our study site, approximately 5% of males are polygynous.

In Michigan, most house wrens returning at the beginning of the breeding season are double-brooded, resulting in a bimodal distribution for the date of nest lining (Figure 3.1). I classified nests with line dates in the first peak as early season nests (those lined prior to 6 June). Nests lined after 6 June were considered late season nests. Most late season nests were second broods for one or both members of the pair, but some new breeders did appear on the study site during the late season nesting period.

Experimental manipulation

Prior to male arrival, I plugged two of the three nest boxes in each territory with rubber stoppers. Therefore, all males settled in single box territories. I checked nest boxes at least every other day during the male and female settlement periods. A territory was considered settled after a male was observed singing near an available nest box for two consecutive nest checks or if nesting material was observed in a nest box.

I manipulated cavity availability in male territories twice during the nesting period. First, I applied a *pre-pairing treatment* within two days of male settlement in order to randomize male quality across treatments, while leaving open the possibility for males to opportunistically vary investment in mate attraction and/or paternal care. I randomly assigned territories to either single cavity or surplus cavity treatments. In single cavity territories, stoppers were maintained in the two plugged nest boxes, whereas in surplus cavity territories, stoppers were removed from the two plugged nest boxes. To control for time effects of male settlement, I paired sites by male settlement date and randomly applied treatments within those pairs. By randomizing treatments across males prior to female settlement, I was able to examine the effect of cavity availability (independent of male quality) on female mate choice and reproductive investment (Chapters 4-5).

In order to look at the effect of cavity availability on male trade-offs between mate attraction and provisioning effort during the incubation and nestling periods, I also needed to randomize differences in female quality across the experimental treatments. Therefore I re-randomized experimental treatments at the onset of incubation (the *post-laying treatment*), again assigning territories to either single or surplus cavity treatments. This second experimental manipulation allowed me to look for an effect of cavity availability on male trade-offs between mate attraction and provisioning effort independent of differences in female condition. For example, suppose that females preferred monogamous males, then higher quality females might preferentially settle in single cavity territories. As a result, males in territories with single cavities might invest less in provisioning, not as a result of cavity availability, but because they were mated to females in better condition that could provision more. By re-randomizing treatments at the onset of incubation, I de-coupled the possible correlation between female condition and cavity availability during the incubation and nestling periods.

After nestlings fledged, I cleaned nesting material out of nest boxes and maintained the 'post-laying' configuration as the 'pre-pairing' treatment for late season nests. At the onset of incubation, I again re-randomized the experimental treatments, just as for early broods.

Provisioning effort and mate attraction

I captured adults prior to provisioning observations using a mist net or box trap, and marked each adult with an aluminum U.S. Fish and Wildlife Service leg band and a unique combination of three color bands. I observed parental provisioning visits for 25-30 minutes in the morning (between 7:00 and 11:00 am) when nestlings were 4-5 days old, 8-9 days old, and 11-12 days old. Brood day (BD) 0 is defined as the day the first nestling in a given nest hatched. Provisioning observations were conducted during fair weather using 10x binoculars mounted on a tripod, taking care to minimize disturbance. Any parental visit to the entrance of the nest box was considered a provisioning trip, regardless of whether food was actually observed.

I recorded male song using a Sony TCM-5000EV or a Marantz PMD221 cassette recorder and a parabolic reflector (Sony PBR-330) with a Calrad super cardioid condenser microphone. Songs consist of a relatively low amplitude introduction section made up of acoustically complex elements followed by a louder terminal section of repeated trill notes (Platt and Ficken 1987; Figure 3.2). Song was recorded in the morning for 20-45 minutes during the incubation period (4-5 days after the post-laying treatment was applied) and again when nestlings were 6-8 days old. If recording conditions were poor, or no song was recorded, males were recorded again on a following day (total recording time never exceeded 45 minutes). I calculated an index of song rate by dividing the number of 15-sec intervals during which a male sang at least once by the total number of recording intervals. This index is a conservative estimate of song rate because it underestimates high song rates (males that sing frequently sing 3-4 times per 15-sec interval). In addition, males that sang little tended to be recorded for shorter periods of time, in which case, the index would overestimate song rates of males that sang little. I counted any vocalization during an interval as a "song." By using counts instead of time spent singing, I avoided problems associated with variable recording conditions in the field, *e.g.*, wind interference or poor recording quality. Hence, songs could be counted from the tape even when precise measurements of song length were not possible.

Avisoft SASLab Pro was used to analyze digitized song recordings. For each recording, I measured the duration of the introductory section for a randomly selected subset of five songs containing a terminal trill section (except for one male that only sang three songs). Song elements separated by less than 0.2 seconds were considered part of the same song. I excluded songs consisting only of "introduction notes" and songs with "introduction notes" inserted into an otherwise continuous trill sequence. Songs for which the beginning or end of the introduction sequence could not be distinguished due to poor recording quality were omitted from the analysis. Males rarely sang during recording sessions at late season nests, and I included only early season nests in song analyses.

Statistical analyses

I used standard tests to analyze frequency data (Sokal and Rohlf 1995): Cochran's Q test for changes in the proportion of males provisioning over time (repeated measures) and Chi-square tests of independence for treatment effects on the proportion of males provisioning. In one case, analysis of treatment effects at late season nests, Chi-square tests of independence were inappropriate due to small sample sizes (*i.e.*, the expected values were less than 5 for some categories). Therefore, I calculated 95% confidence intervals around the log odds ratio for the difference in the proportion of males provisioning in surplus versus single cavity territories in order to determine whether the log odds ratio differed from zero (Sokal and Rohlf 1995). I analyzed provisioning rates and song variables (repeated measures) using profile analysis with MANOVA (PROC GLM) in SAS v.8.1 (SAS Institute Inc. 2000).

Data were transformed where necessary to improve normality of residuals and reduce heteroscedasticity for parametric analyses. Provisioning rates were standardized to a 30-minute observation period for analysis. The pre-pairing treatment and pre-pairing × post-laying treatment interaction were non-significant in analyses of provisioning and song rates ($P > 0.2$), indicating that prior treatment history had no effect on these variables after the post-laying treatment was applied. Therefore, I included only the post-laying treatment in those analyses. There was a tendency for the pre-pairing treatment to affect introduction length of songs, so both treatments and the interaction were included in that particular analysis.

Polygynous males and males that lost control of a "surplus" nest box to intruding males were excluded from analyses because both occurrences disrupted the surplus cavity treatment (*i.e.*, such males were unlikely to have opportunities to attract additional females). Polygyny occurred seven times over the two years of the study. Split territories occurred five times in 2002 only. Males that returned between years were assumed to be independent data points for analysis. Eleven males produced at least one clutch during both years. Analyses including returning males are presented, but I also ran these analyses with each male included only once in the data set (randomly selecting which entry to include). Unless noted, these more conservative tests yielded the same results. Means ± se are presented (means for transformed data are back-transformed into original units).

RESULTS

Parental investment

At early season nests, the proportion of males provisioning was affected by nestling age (surplus cavities: $Q = 18.0$, $df = 2$, $P < 0.001$; single cavities: $Q = 24.7$, $df = 2$, $P < 0.0001$; Table 3.1). Almost all males provisioned on BD 4 (97%, the two males that did not occurred in surplus cavity territories). Fewer males provisioned when nestlings were older, but there were no differences between territories containing surplus and single cavities (BD 8: $X^2 = 0.012$, $df = 1$, $P > 0.9$; BD 12: $X^2 = 0.05$, $df = 1$, $P > 0.8$). At late season nests, the proportion of males that provisioned was high (> 90%) and was not related to age of nestlings (surplus cavities: all males provisioned on all days; single cavities: $Q = 0.5$, df = 2, $P > 0.7$). There was no difference in the proportion of males provisioning on BD 12 between males with surplus and single cavities in their territories at late season nests (log odds ratio with 95% confidence limits: 2.2 ± 2.98). Combining treatments, more males provisioned at late season nests than early season nests on BD12 ($X^2 = 24.0$, $df = 1$, $P < 0.0001$). Of males that provisioned young for the entire nestling period, there were no differences in per-nestling provisioning rates between treatments or between broods, but there was a significant effect of nestling age on paternal provisioning rates (Table 3.2; Figure 3.3).

Males delivered only a single prey item during virtually all of the provisioning trips we observed. We did not measure prey items, but the vast majority of prey items were small (less than one bill length long) and varied little between territories. It is unlikely that there were any significant differences between treatments in the amount of food males delivered per provisioning trip.

Investment in paternal care at first brood nests might have affected territory retention or the likelihood of rearing a second brood. Males that provisioned first brood young on brood day 12 were not more likely to retain the same territory for second broods than males that did not provision young on brood day 12 ($X^2 = 1.1$, $df = 1$, $P > 0.3$; Table 3.3). Seventy-six percent of males that did not provision first brood young reared second broods, whereas only 56% of males that provisioned first brood young reared second broods, but these differences were not significant ($X^2 = 2.6$, $df = 1$, $P < 0.11$; Table 3.3). Polygynous males were included in this analysis, but all unsuccessful first brood nests were excluded.

Mate attraction

At early season nests, males with surplus nest boxes in their territories had a higher song rate index than males with single nest boxes in their territories (Table 3.4; Figure 3.4). This difference was primarily due to males in territories containing single nest boxes reducing the amount of time spent singing between the incubation and nestling periods, whereas males in territories containing surplus nest boxes maintained similar song rates between periods. In the nestling period, males with surplus cavities sang during approximately 40% of recording intervals versus approximately 20% of recording intervals for males with single cavities in their territories.

Cavity availability during the incubation and nestling periods had no effect on the introduction length of male songs (Table 3.5; Figure 3.5), although there was a tendency towards an historical effect of the pre-pairing treatment. Males with surplus nest boxes in their territories *before* the incubation period tended to sing longer introduction sequences during the incubation period than males with single nest boxes in their territories before the incubation period. The repeated measures analysis included only nests for which I was able to record song during both the incubation and nestling periods, and therefore excluded many nests that failed after the incubation recording. In order to utilize the entire data set, I restricted the analysis to the incubation period. Again, there was a non-significant increase in introduction length for males with surplus cavities in their territories *before* the incubation period (ANOVA: pre-pairing treatment, $F_{1,64} = 3.0$, $P < 0.09$, overall model ns; means: surplus cavities = 0.85 ± 0.069 sec, single cavities = 0.67 ± 0.076 sec).

DISCUSSION

Whether a male should invest in parental care or mate attraction depends on the relative fitness benefits of current and future reproduction. For cavity nesters, opportunities to attract additional mates are limited by both mate availability and the availability nest sites. I found that already-mated males with surplus cavities in their territories sang more after pairing than mated males with a single (occupied) nest site in their territories. Adding surplus cavities to a male's territory not only increased a male's opportunity to attract additional mates, but might also have increased the perceived quality of the territory, resulting in a shift in investment towards mate attraction effort.

The observed differences in song rate were much greater during the nestling period than the incubation period. Males with surplus cavities sang at high rates during both the incubation and nestling periods, whereas males with single cavities sang at high rates during the incubation period, but decreased song rates during the nestling period.

Since male house wrens do not incubate and the risk of cuckoldry is reduced during the incubation period, opportunities for extra-pair matings might be high during this time regardless of cavity availability. If opportunities for extra-pair matings decrease as the nesting cycle progresses (*e.g.*, if fewer neighboring females are fertile after eggs hatch), then males with single nest boxes in their territories would gain little by advertising during the nestling period, but males with surplus cavities might still have opportunities to attract secondary females. An alternative explanation for persistently high song rates among males with surplus cavities in their territories might be territorial defense against intruding males trying to usurp nest sites. In a few territories (5 cases in 2002 only), males did lose control of one of their surplus nest boxes to unmated males. Generally this phenomenon occurred towards the end of the nestling period of first broods or during second broods. But since the primary reason for defending surplus cavities is to attract additional females, defense of surplus cavities would still serve to increase mating opportunities, even if the song were not specifically directed at females.

Little is known about the specific aspects of male song that females might use in assessing mates. E.D. Kennedy and J.M. Lueken (pers. comm.) observed that unmated males sing longer introduction sequences than mated males. I did not measure the introduction sequence of songs of unmated males but looked for differences between advertising and non-advertising mated males (*i.e.*, males with surplus versus single cavity territories). I did not find a difference in the length of the introduction sequence between mated males with single versus surplus cavities in their territories during the incubation and nestling periods. However, there was a tendency for males with surplus nest boxes in their territories *prior* to the incubation period to sing longer introduction sequences, particularly during the incubation period. Perhaps males with surplus cavities in their territories prior to the incubation period sang longer introduction sequences earlier in the nesting cycle and switched to shorter introductions during the late incubation or nestling periods.

Even though males with surplus cavities in their territories exhibited higher song rates during the nestling period, there were no differences in provisioning rates between males with surplus and single nest boxes in their territories. This pattern suggests that either the costs of mate attraction are low for males with surplus boxes or that the benefits associated with increased parental effort are low for males with single nest boxes in their territories. Smith (1995) found that male European starlings with a surplus nest box added to their territories increased mate attraction effort at the expense of paternal investment during the incubation period when mate attraction opportunities were high, but not during the nestling period when mate attraction opportunities were low. It is unlikely that low mate attraction opportunity accounts for the failure to observe a trade-off between mate attraction and parental investment during the nestling period in this study. New females were frequently observed on the study site during late season broods and were presumably also present during the nestling period of early season broods. Considering this observation, the fact that polygyny did not occur more often is surprising (5-10% of males with surplus boxes were polygynous), but one possible explanation is that the three nest boxes within a territory were spaced relatively close together, and resident females may have been able to exclude prospecting females (Slagsvold and Lifjeld 1994).

Males may have faced different, unmeasured costs of increased mate attraction opportunity, such as increased territorial defense of surplus cavities, or trade-offs might have occurred earlier in the nestling period. For house wrens, the most critical stage of paternal care may be during the earliest part of the nestling period when females are brooding young (Johnson *et al.* 1992). Male removals later in the nestling period (after brood day 4) decrease reproductive success only under unfavorable environmental conditions (Bart and Tornes 1989). Therefore, the trade-off between mate attraction and paternal care might be most apparent during the first few days after eggs hatch. In this study, the earliest provisioning observations occurred on brood day 4, and male song was recorded on brood days 6-8. If paternal care were relatively unimportant later in the nestling period, it would not be surprising to find similar provisioning rates between males that were investing in mate attraction and those that were not.

Regardless of cavity availability, male investment in provisioning at early season nests was lower than at late season nests (see also Dubois and Getty 2003). Virtually all males provisioned at late season nests, whereas fewer than one-half of males provisioned for the entire nestling period at early season nests. After nestlings fledge, one or both parents generally care for the young until they reach independence (Kendeigh 1941). Males that care for young after fledging are more likely to lose their territory and less likely to have a second brood (Bart 1990). It is possible that males that desert towards the end of the nestling period are less likely to end up caring for fledglings. Therefore, the costs of investing in parental care at early season nests could be high for males of double-brooded species, regardless of polygyny potential, if male care towards the end of the nestling period during the first brood decreases the probability of rearing a second brood.

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Table 3.1. Proportion of males provisioning nestlings in territories containing surplus and single cavities (post-laying treatment) at early and late season nests.[a]

	Early season nests		Late season nests	
Brood day	Surplus cavities (n = 25)	Single cavities (n = 33)	Surplus cavities (n = 22)	Single cavities (n = 26)
4	0.92	1.00	1.00	0.88
8	0.68	0.67	1.00	0.88
12	0.44	0.45	1.00	0.85

[a] Nests with incomplete data were excluded.

Table 3.2. Repeated measures analysis of paternal provisioning rates per nestling on brood days 4, 8, and 12 for males in territories containing surplus nest boxes and single nest boxes during the nestling period (post-laying treatment) at early and late season nests. Only males that provisioned for the entire nestling period were included.[a]

A. MANOVA (profile analysis) of within subject effects. Pillai's trace values are presented.

Source	Num df	Den df	Value	*F*	*P* > *F*
Brood day (time)	2	62	0.14	5.2	0.01
Brood day × Season	2	62	0.041	1.3	0.2
Brood day × Treatment	2	62	0.010	0.33	0.7
Brood day × Season × Treatment	2	62	0.016	0.51	0.6

B. Repeated measures ANOVA of between-subject effects.

Source	df	MS	*F*	*P* > *F*
Season	1	0.056	0.40	0.5
Treatment	1	0.001	0.01	0.9
Season × Treatment	1	0.016	0.11	0.7
Error	63	0.14		

C. ANOVAs on each of the contrasts for time differences between successive observations. Only the brood day effect is shown, others were non-significant.

Source	df	MS	*F*	*P* > *F*
Contrast variable: BD8 – BD4 Brood day	1	0.002	0.04	0.8
Error	63	0.063		
Contrast variable: BD12 – BD8				
Brood day	1	0.82	7.03	0.01
Error	63	0.12		

[a] Data square root transformed. Nests with incomplete data were excluded.

Table 3.3. Number of male house wrens retaining the same territory for first and second broods, switching territories between broods, and losing territories in relation to paternal provisioning at first brood nests. Only males with successful first brood nests were included.

	Reared a second brood		
		Lost territory	
First brood	Retained territory	Switched territories	No known second brood
Provisioned on brood day 12	14	1	12
Did not provision on brood day 12	19[a]	3	7

[a] Includes a polygynous male that retained his territory for a secondary female and then lost it to another male.

Table 3.4. Repeated measures analysis of male song rate during the incubation and nestling periods for males in territories containing surplus nest boxes and single nest boxes (post-laying treatment) at early season nests.[a]

A. MANOVA (profile analysis) of within subject effects. Pillai's trace values are presented.

Source	Num df	Den df	Value	*F*	$P > F$
Period (time)	1	46	0.035	1.7	0.2
Period × Treatment	1	46	0.083	4.2	0.05[b]

B. Repeated measures ANOVA of between-subject effects.

Source	df	MS	*F*	$P > F$
Treatment	1	0.22	6.1	0.017
Error	46	0.036		

[a] Nests with incomplete data were excluded.

[b] Period × Treatment effect not significant ($P > 0.13$) with more conservative analysis which included returning males only once in the data set.

Table 3.5. Repeated measures analysis for the length of the introduction sequence of male songs during the incubation and nestling periods for male house wrens in territories containing surplus nest boxes and single nest boxes during the pre-pairing treatment (PPT) and post-laying treatment (PLT) periods at early season nests.[a]

A. MANOVA (profile analysis) of within subject effects. Pillai's trace values are presented.

Source	Num df	Den df	Value	*F*	*P* > *F*
Period (time)	1	43	0.015	0.65	0.4
Period × Pre-pairing treatment	1	43	0.067	3.1	0.09
Period × Post-laying treatment	1	43	0.042	1.9	0.18
Period × PPT × PLT	1	43	0.020	0.90	0.3

B. Repeated measures ANOVA of between-subject effects.

Source	df	MS	*F*	*P* > *F*
Pre-pairing treatment	1	0.60	3.1	0.08
Post-laying treatment	1	0.13	0.69	0.4
PPT × PLT	1	0.034	0.17	0.6
Error	43	0.19		

[a] Nests with incomplete data were excluded.

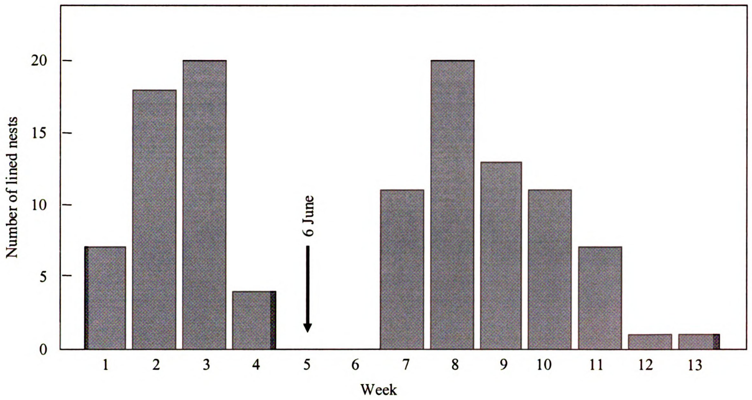

Figure 3.1. Frequency distribution of nest lining dates in 2002. In both years of the study, 6 June was used as the division between early and late season nests. The first nest was lined on 30 April.

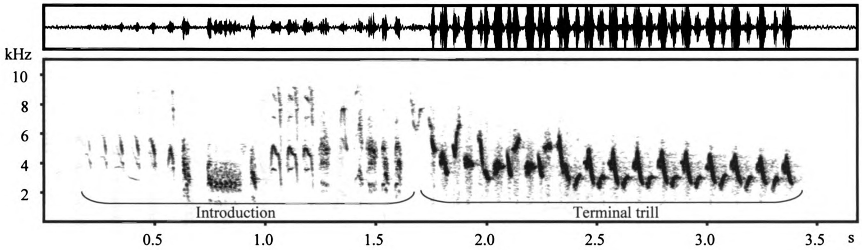

Figure 3.2. Spectrogram of a male house wren song. The softer introduction sequence consists of complex elements and is followed by a louder terminal trill consisting of repeated elements. Amplitude is indicated in the box above the spectrogram.

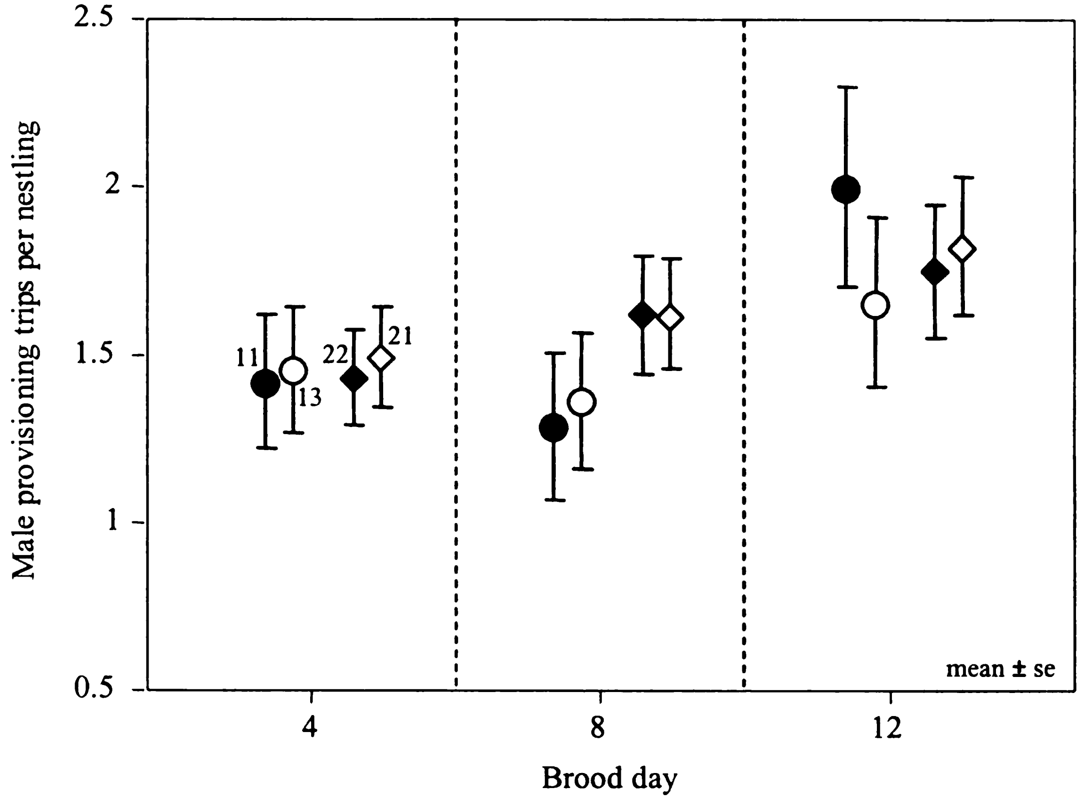

Figure 3.3. Average paternal provisioning rates per nestling during 30-minute observation periods for males in territories containing surplus nest boxes (solid symbols) and single nest boxes (open symbols) during the nestling period. Early season nests indicated by circles, late season nests indicated by diamonds. Only males that provisioned for the entire nestling period were included. Nests with incomplete data were excluded, therefore samples sizes (indicated) are the same across brood days. There was a significant brood day effect (*P* < 0.01).

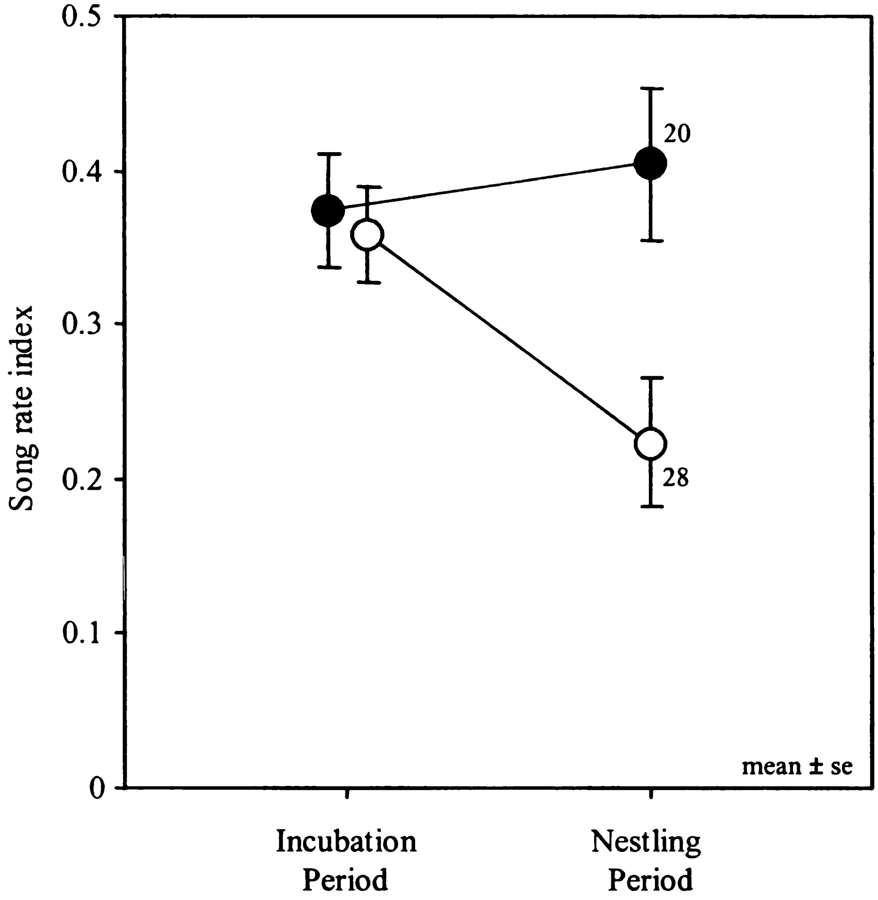

Figure 3.4. Song rate index for males in territories containing surplus cavities (solid symbols) and single cavities (open symbols) during the incubation and nestling periods at early season nests. The treatment main effect was significant ($P < 0.02$). The interaction was marginally significant ($P = 0.05$). Nests with incomplete data were excluded. Sample sizes are indicated.

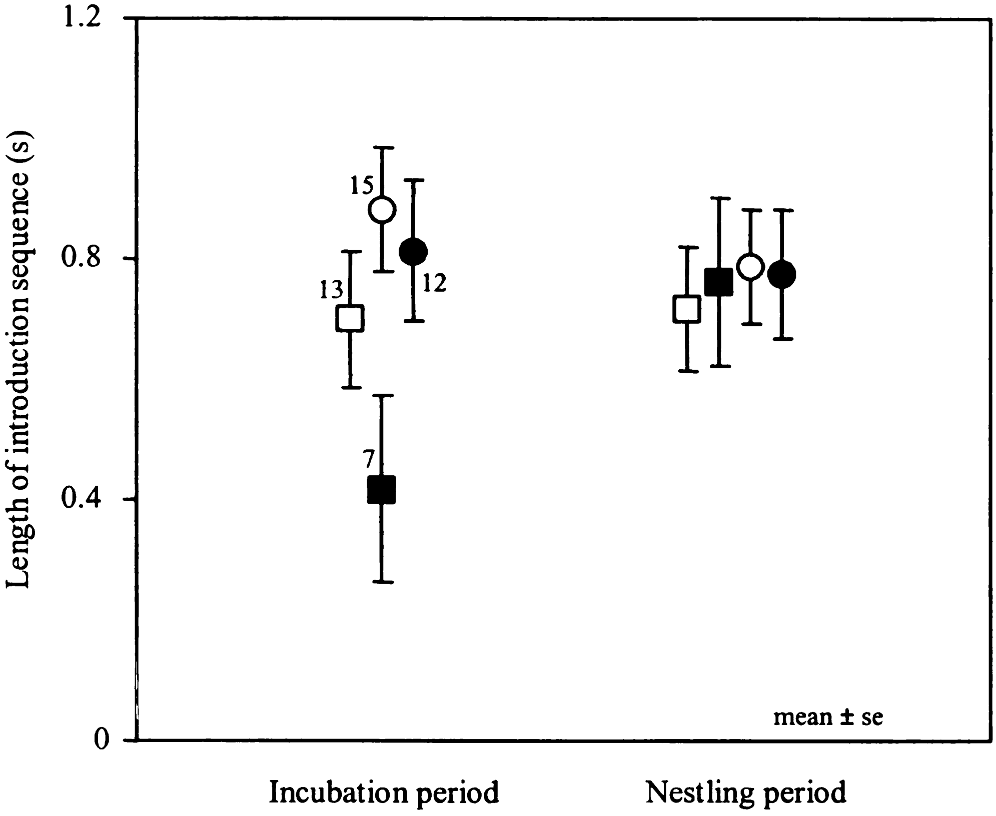

Figure 3.5. Length of the introduction sequence for songs of male house wrens in territories containing surplus (solid symbols) and single (open symbols) cavities in their territories during the incubation and nestling periods. Pre-pairing treatment is indicated by circles (surplus cavities) and squares (single cavities). Means were not significantly different. Nests with incomplete data were excluded, therefore samples sizes (indicated) are the same for the incubation and nestling periods.

CHAPTER 4

MATERNAL INVESTMENT BY FEMALE HOUSE WRENS DEPENDS ON TERRITORY CHARACTERISTICS RELATED TO MALE MATE ATTRACTION OPPORTUNITY

with Thomas Getty

ABSTRACT

Whether a high quality mate will invest in mate attraction at the expense of parental care sets up a potential conflict for females of polygynous species. For cavity-nesting species unable to excavate their own cavities (secondary cavity nesters), a male's opportunity to become polygynous is limited by the availability of cavities within his territory. When male parental care affects reproductive success, then females might assess male opportunity for polygyny when selecting mates and prefer males with low polygyny potential. Alternatively, male competition over territories with high polygyny potential (*i.e.*, multiple cavities) might result in a positive relationship between male quality and cavity availability within his territory. If males vary in their ability to provide benefits to females, either direct or indirect, then females should demonstrate preferences for males providing benefits and adjust reproductive investment in response the quality of their mate. We experimentally manipulated cavity availability independently of male quality and found that first brood females mated to males with multiple cavities in their territories laid larger clutches than females mated to males with single cavities in their territories. Cavity availability had no effect on the timing of nest lining (our index of the timing of female settlement) or on female site fidelity between early and late season broods.

INTRODUCTION

Darwin (1871) identified two components of sexual selection that contribute to variance in reproductive success among individuals, (1) intrasexual competition, generally males competing for access to females, and (2) intersexual selection, where females, often the choosy sex, prefer some males over others. Numerous studies have shown that females can gain a variety of benefits from mate choice (reviewed in Andersson 1994). Females might choose mates on the basis of characters that signal direct benefits such as territory quality (Pärt and Qvarnström 1997), paternal care (Hill 1991), and/or indirect benefits such as genetic quality (Møller 1994). When males both defend territories and provide parental care, male competition over access to high quality territories may facilitate female choice of high quality mates (Qvarnström *et al.* 2000).

For cavity-nesting species unable to excavate their own cavities (secondary cavity nesters), cavity availability limits opportunities for polygyny. Experimental studies have demonstrated that rates of polygyny can increase with the number of cavities available in a male's territory (*e.g.*, Johnson and Kermott 1991a, Petit 1991). When the number of defendable cavities differs between available territories, we might expect competition among males to lead to higher quality males defending territories with greater numbers of cavities. If males also provide direct benefits to females, such as parental care, trade-offs between parental investment and mate acquisition set up a possible conflict between male and female interests in reproductive investment (Davies 1989, Lifjeld and Slagsvold 1991, Kempenaers 1995, Smith and Sandell 1998).

Many studies have demonstrated costs of polygyny for females, such as reduced paternal assistance and lower reproductive success (*e.g.*, Lifjeld and Slagsvold 1989, Pinxten and Eens 1994, Kempenaers 1995, Sandell *et al.* 1996). Even prior to the acquisition of a secondary female, first-mated females may face reduced paternal care from males attempting to attract additional mates. For example, already-mated male European starlings (*Sturnis vulgaris*) with a surplus cavity added to their territory increased mate attraction effort and contributed less to incubation than did males with only a single cavity (Smith 1995). The apparent aggressiveness of resident females towards female intruders in many species with bi-parental care suggests that females may engage in a form of mate guarding to insure male parental investment (Slagsvold and Lifjeld 1994). In a Wyoming population of house wrens (*Troglodytes aedon*), females sometimes sang when their mates engaged in courtship activities with additional females (Johnson and Kermott 1990), perhaps to discourage secondary females from settling.

We investigated reproductive investment decisions in a secondary cavity nester, the house wren. On our study site in southwest Michigan, house wrens are frequently double-brooded, and within each brood approximately 5% of males are polygynous. In other populations, male parental care during the nestling period has been shown to affect female reproductive success (Johnson *et al.* 1992), and reduced paternal care contributes to lower reproductive success of secondary females (Johnson *et al.* 1993). Male house wrens frequently advertise for secondary females after pairing (Johnson and Kermott 1991b). Females might therefore be sensitive to male mate acquisition opportunity when selecting a mate. We tested this hypothesis by manipulating cavity availability in male house wren territories. We evaluated the effect of increased mate attraction opportunity on parental investment by both males and females. Specifically, we asked whether cavity availability affects (1) female mate choice or investment in clutch size and (2) male parental care and mate attraction effort. Here we report our results on female mate choice and maternal investment.

METHODS

Cavity manipulation

During April through August of 2001 and 2002, we conducted a cavity manipulation experiment on a population of house wrens at the Kellogg Biological Station's Lux Arbor Reserve in Barry County, Michigan (see Chapter 3 for a complete description of the study site and experimental manipulation). We controlled male access to multi-cavity territories by manipulating nest boxes within male territories after male settlement but prior to pairing. Prior to male settlement, all territories contained three nest boxes, but males had access to only a single nest box. The remaining nest boxes had rubber stoppers placed in the entrance holes. After male settlement, but before female settlement, we randomly assigned territories to single or surplus cavity treatments by removing rubber stoppers from the nest boxes in surplus cavity territories (the pre-pairing treatment). Consequently, females chose among males with a single cavity or multiple cavities in their territories at settlement. This treatment was maintained through the pairing and egg-laying periods, at which point we re-randomized treatments across territories (the post-laying treatment, see Chapter 3).

In this paper we focus only on maternal reproductive investment decisions, addressing mate choice, clutch size, and site fidelity between broods. We used the date of female nest lining as an index of pairing date and calculated the number of days a male remained unmated as the time between male settlement and nest lining. The number of days a male remained unmated was used as an indicator of female mate choice. In cases where we missed the first sign of lining and found a lined nest containing an egg on a subsequent visit, we used the day between our nest checks as the nest-lining date. We checked nests every other day to determine clutch size, and used the total number of eggs laid as our measurement of clutch size, even if clutch size was reduced during the incubation period. We excluded re-nests that occurred within the early or late season breeding periods from our analyses. Therefore the data set included a maximum of one early season nesting attempt and one late season nesting attempt for each individual female. Nests that failed partway through the nesting period were included in analyses based on data collected before the failure. Breeding females were captured during the late incubation periods or nestling periods. In order to assess the relative size of females settling in single and surplus cavity territories, we collected the following standard morphological data at the time of capture: length of exposed culmen, tarsus length, and unflattened wing chord.

Statistical analyses

When data were approximately normally distributed we used parametric statistical methods (ANOVAs with type-III sums of squares). We transformed data when necessary to improve normality of residuals. All parametric analyses were conducted in SAS v.8.1 (SAS Institute Inc. 2000). When interactions were significant, we used the SLICE command in lsmeans to test for differences between treatments within a brood. Data with moderate violations of the assumptions of parametric tests (*e.g.*, in cases in which homogeneity of variance or normality of residuals could not be improved with data transformations) were also analyzed with non-parametric equivalents. For settlement and clutch size analyses, error variances between years were homogeneous (Fmax test, Kuehl

1994) and we combined data from both years. When there were significant differences in dependent variables between years, we standardized data within each year to a standard normal distribution before combining the data. Standardizing data within each year simplified our models by eliminating the Year effect and interactions with Year from our models. For settlement and clutch size analyses, we treated females returning between years as independent data points. Within a year, approximately 20-30% of adults were returning breeders. On our study site, fourteen females attempted at least one brood in both years. Analyses including returning females are presented, but we also ran these analyses with each female included only once in the data set (randomly selecting which entry to include). In all cases, these more conservative tests yielded the same results.

We analyzed female size data at early season nests only. We used MANOVAs, combining years but including each female only once in the analysis (randomly selecting which entry to use for returning females). We excluded exposed culmen length from the MANOVA because the sign of the correlation between culmen length and the other dependent variables differed among groups in some cases, violating key assumptions of the analysis (Scheiner 1993). Therefore, we analyzed culmen length separately with a univariate test. Excluding culmen length had no effect on significance of the model. For all data, means ± se are reported.

RESULTS

Female settlement

We found no differences in the date of nest lining (our index of the timing of female settlement) between females settling in territories with three nest boxes or a single nest box. We considered only the first nesting attempt of the season for each female.

The time a male remained unmated, that is the number of days between male settlement and the date of nest lining, did not differ for males with surplus nest boxes in their territories and males with a single nest box in their territories (surplus = 10.9 ± 1.06 days vs. single = 11.6 ± 1.05 days, untransformed data; ANOVA: $F_{1,81} < 0.01$, $P > 0.9$, data log transformed and standardized within years; Figure 4.1). In addition, there were no differences in the Julian date of nest lining between surplus cavity and single cavity territories (ANOVA: $F_{1,82} = 0.48$, $P > 0.4$, data standardized within years).

Females settling in territories containing surplus cavities were not larger than females settling in single box territories at early season nests (MANOVA including tarsus and wing chord: Pillai's trace value 0.044, $F_{2,55} = 1.3$, $P > 0.2$; ANOVA for exposed culmen: $F_{1,59} = 1.84$, $P > 0.17$; Table 4.1). We could not age females but could identify returning breeders banded as adults the previous year (≥ 2 years old) and first year breeders banded as nestlings the previous year. We had no information about the age of unbanded breeders. For analysis, we assumed that unbanded breeders were young birds and lumped them with first year breeders. We found no difference in the relative age of females mated to males with surplus nest boxes versus single nest boxes at early season nests ($X^2 = 0.14$, df = 1, $P > 0.6$; Table 4.2).

Clutch size

We included double-brooded females in our experiment. Although we re-randomized treatments across experimental sites during the incubation period, thus giving each territory an equal probability of being assigned to each treatment during the late season, the presence of double-brooded females in our data set (included twice per year) could reduce independence between data points. In fact, clutch sizes of double-brooded females were correlated regardless of the experimental treatment ($r = 0.37$, $P < 0.02$, clutch sizes standardized within each Year × Season combination). We addressed non-independence of clutch sizes of double-brooded females in two ways. First, we looked only at early season nests. This maximized our sample sizes for early season nests while excluding any replicated second brood females. At early season nests, females mated to males with surplus cavities laid larger clutches than those mated to males with single cavities (surplus = 6.9 ± 0.12 eggs vs. single = 6.5 ± 0.13 eggs, untransformed data; ANOVA: $F_{1,72} = 6.6$, $P < 0.02$, data standardized within years; Wilcoxon two-sample test: $t_s = 2.5$, $P < 0.02$; Figure 4.2). Second, in order to include both early and late season nests in our model, we randomly assigned double-brooded females to either the first or second brood, thereby including each female only once per year in the data set. In doing so we decreased the sample sizes in each Treatment × Season combination and thus reduced the power to detect treatment differences within each brood. There was a significant Treatment × Season interaction (ANOVA: treatment, $F_{1,99} < 0.01$, $P > 0.9$; season, $F_{1,99} = 7.6$, $P < 0.01$; treatment × season, $F_{1,99} = 6.1$, $P < 0.02$, data standardized within years). For early season nests, we observed similar results to our previous analysis, females mated to males with surplus cavities tended to lay larger clutches than females mated to males with single cavities (surplus = 6.9 ± 0.16 eggs, $n = 28$ vs. single = 6.5 ± 0.17 eggs, $n = 26$, untransformed data; differences between treatments for early season nests: $P < 0.07$, data standardized within years). At late season nests, differences between treatments were not significant (surplus = 6.1 ± 0.19 eggs, $n = 19$ vs. single = 6.5 ± 0.15 eggs, $n = 30$, untransformed data; differences between treatments for late season nests: $P > 0.1$, data standardized within years).

Site fidelity

We looked at whether females mated to males with three box territories were more likely to stay on the same territory for second broods than females mated to males with one box territories. We included only successful early season nests because we suspected that females re-nesting after a failed attempt might use different decision rules than females that produced a successful first brood. Females classified as having left the territory after a first brood either produced a second brood on another territory or disappeared from our study site during the late breeding season. Since the decision to stay or leave a territory for the second brood occurred after the second experimental manipulation, we classified females into four treatment groups (Table 4.3). There were no differences in the proportion of females staying in the same territory for second broods among treatment groups ($X^2 = 2.3$, df = 3, $P > 0.5$). In fact, most females attempting both early and late season nests on the study site remained on the same territory for both broods (34 out of 43 females).

DISCUSSION

Models of polygyny often focus on the decisions of secondary females (*i.e.*, when a female should choose an already-mated male versus an unmated male). When polygynous males divide parental care between nests, the decision of the secondary female can also affect the first-mated female's reproductive success. Therefore, early settling females might be able to increase reproductive success if they could assess male polygyny potential. In the case of secondary cavity nesters, cavity availability provides a potential indicator of a male's opportunity to attract additional mates.

For house wrens, paternal provisioning rates do not differ between the nests of monogamous and primary females (Czapka and Johnson 2000). Polygynous males preferentially care for primary nests, and secondary females suffer most of the costs of mate-sharing (Johnson *et al.* 1993). In our study population, cavity availability does not appear to affect paternal provisioning rates among males with a single mate (Chapter 3). Thus, increased opportunities for polygyny might not negatively affect the success of first-mated females. In fact, our data suggest the opposite: during the early part of the breeding season, females mated to males with surplus cavities in their territories laid larger clutches than females mated to males with a single cavity in their territories. Our experimental manipulation randomized mating opportunity across differences in male quality. Consequently, females settling in three box territories did so on the basis of cavity availability, not on some aspect of male quality that might be correlated with cavity availability under natural conditions, and the observed differences in clutch size were not in response to male quality or condition per se. We hypothesize two possible scenarios for increased maternal investment among females mated to males with surplus cavities in their territories, both of which suggest that females expect to benefit from mating with males with multiple cavities in their territories.

First, mate choice could result in non-random pairing with respect to cavity availability. High quality females and/or females in good condition might have greater access to attractive males or be more choosy in mate selection, thus giving more attractive males access to higher quality mates (the differential access hypothesis: Burley 1986). For example, in a lekking species, black grouse (*Tetrao tetrix*) females in better condition paired with dominant males (Rintamäki *et al.* 1998). If females in better condition or of higher quality are able to produce larger clutches, this could explain the observed differences in clutch size between females mated to males with surplus versus single cavities in their territories. Females mated to males with surplus cavities in their territories were not larger than females mated to males with single cavities, but size alone might not be a good predictor of female quality and/or condition.

Second, females mated to attractive individuals might contribute greater than average parental investment to reproduction (the differential-allocation hypothesis: Burley 1986). Females mated to high quality males have been shown to adjust reproductive allocation to produce more eggs (Petrie and Williams 1993), lay larger eggs (Cunningham and Russell 2000), deposit higher levels of testosterone in eggs (Gil *et al.* 1999), or participate in fewer extra-pair copulations (Burley *et al.* 1994). In order to experimentally demonstrate differential allocation, we would have had to manipulate cavity availability after pairing or have randomly allocated mates (Sheldon 2000). As well as being logistically difficult in a field study, random allocation of mates would have prevented us from assessing patterns of female settlement. The second (post-laying) treatment manipulated cavity availability after pairing, but did so after female investment in clutch size had been determined. As a result, we cannot assess the relative contributions of female condition and differential allocation to investment in clutch size, nor are they mutually exclusive.

Both of these hypotheses suggest that cavity availability might be a male status symbol that females use in assessing mates. Alternatively, females might choose territories for the surplus cavities themselves if, for example, the availability of additional nest sites allowed faster renesting after nest failure. Plissner and Gowaty (1995) found preferences for multi-box territories in Eastern bluebirds (*Sialia sialis*) before the onset of breeding activity (mostly males and females visiting together), but no effect on reproductive success. As a result of our experimental approach, on average, males holding one box and three box territories should have been equivalent in terms of condition and/or quality, but we do not know how they responded to cavity availability after the pre-pairing experimental manipulation. We did not assess courtship intensity among males but assumed that mate attraction effort by *unmated* males would be similar regardless of cavity availability. However, at least in fishes, there is some evidence that resource attractiveness can affect mate selectivity by males. Male beaugregory damselfish (*Stegastes leucosticus*) provided with attractive breeding territories invested more in courtship and adjusted courtship intensity to female quality (Itzkowitz and Haley 1999). We did observe prospecting females in both single cavity and surplus cavity territories and have no a priori reason to believe that unmated males with surplus cavity territories invested more in mate attraction than unmated males with single cavity territories.

Timing of female settlement

Although female house wrens mated to males with surplus cavities laid larger clutches than females mated to males with a single cavity during early nests, we did not observe differences in the timing of female settlement. If females in better condition were able to acquire "preferred" males or territories (here those with three nest boxes), we would have expected males with surplus nest boxes in their territories to pair earlier than males with single nest box territories. One possibility is that nest building activities such as nest lining are a poor index of settlement date (Stutchbury and Robertson 1987, but see Dunn and Hannon 1992). Historically, the primary female contribution to nest building in house wrens has been thought to be the lining of the nest shortly after pairing (Kendeigh 1952, McCabe 1965), but recent evidence suggests that females may contribute significantly to nest building prior to lining of the nest (Alworth and Scheiber 2000). If females differ in their contribution to nest building, then the correlation between settlement date and line date might be weak. In addition, if older or better condition females are able to displace resident females after pairing (*e.g.*, Alworth and Scheiber 1999) then our measure of the timing of female settlement would not be good indicator of either female quality or mate choice preferences. We were rarely able to identify individual females before the start of egg-laying, and we could have missed such displacements had they occurred.

Females might be prevented from actively displaying preferences among males during the settlement period if there are high costs associated with continued mate search. For example, competition among females for access to unmated males might result in females losing opportunities to pair as first-mated females if they extend their search effort. Costs of mating as a secondary female can be quite high in terms of reduced male assistance in rearing young (Johnson *et al.* 1993). Additionally, females might use cavity availability as only one of several components in assessing mates. For example, female sedge warblers (*Acrocephalus schoenobaenus*) use both song and territory cues in assessing mates (Buchanan and Catchpole 1997). Our experimental design would have de-coupled any correlations between cavity availability and male quality that could occur under natural conditions. In order to examine this possibility, we set up a modified replicate of the experiment in 2003, in which we assigned territories to one or three box treatments prior to male arrival. Males had access to both one box and three box territories at the time of settlement, but we found no preferences among males for territories containing multiple nest boxes, nor did we find a significant difference in date of nest lining between treatments (unpublished data). Males with surplus cavities in their territories tended to provision more than males with single cavities (38% vs. 26% of provisioning trips were by males), but differences were not significant (unpublished data). Under natural conditions, nest site quality would also vary between territories. In a nest box study, Johnson and Searcy (1993) found that female house wrens choose mates at least partly on characteristics of the nest site. On our site, we installed identical nest boxes in all territories. If females used cavity quality, rather than number of cavities, as the major criterion in settlement decisions, we would not expect differences in the timing of female settlement among surplus and single cavity territories containing identical nest boxes. Post-pairing reproductive investment decisions might still be based on cavity availability.

Site fidelity between broods and late season nests

Cavity availability had no effect on the likelihood that a female would breed in the same territory for a second brood. Most double-brooded females retained the same territory for second broods even though treatments had been randomly re-assigned to territories prior to initiation of the second brood. Possibly, females did not reassess territory and/or mate quality between broods, or they used different factors in settlement and reproductive investment decisions for late season broods. In an Illinois population of house wrens, mate switching was unrelated to reproductive success of first broods (Drilling and Thompson 1991). Mate switching and territory retention may be more a consequence of which parent has the first opportunity to desert the young and begin a subsequent breeding attempt.

At late season nests, we found no between-treatment differences in clutch size, but this could be due to the timing of our second experimental manipulation. We re-randomized treatments during the incubation period of early broods and carried that treatment through nest initiation and the laying period of second broods. As a result, females that maintained the same territory for both broods had an equal probability of being assigned to either treatment for the second brood. If the differences in clutch size between treatments during early season nests resulted from differences in female condition, then our re-randomization of treatments during the laying period decoupled any potential correlation between treatment and female condition during late season broods. Double-brooded females laying large first brood clutches were more likely to lay large second brood clutches regardless of whether they switched treatments for the second brood. This suggests that female condition contributes, at least in part, to observed differences in early season clutch sizes between treatments, but does not exclude the possibility that differential allocation after pairing is also occurring. Further experiments are necessary to determine the relative contributions of pre-pairing (differential access) and post-pairing (differential allocation) decisions to maternal reproductive investment in this system.

In conclusion, our results show that female house wrens mated to males with surplus cavities in their territories laid larger early season clutches than females mated to males with only a single cavity in their territories, suggesting that female house wrens use cavity availability in male territories to assess male quality. Higher quality males might have greater access to territories containing multiple nest sites; therefore female choice might be based on indirect benefits. Alternatively, higher quality males able to secure territories with surplus nest sites under natural conditions might also contribute more paternal care, thus providing females with direct benefits. Both scenarios could result in female preferences for males with surplus cavities in their territory and lead to differences in clutch size between males with surplus and single cavities in their territories.

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Table 4.1. Morphological measurements for females settling in territories containing surplus and single cavities at the beginning of the breeding season.

Morphological variable	Surplus box territory	Single box territory
Tarsus length (mm)[a]	19.6 ± 0.11	19.8 ± 0.12
Unflattened wing chord (mm)[a]	48.8 ± 0.31	49.3 ± 0.32
Exposed culmen length (mm)[b]	12.4 ± 0.12	12.1 ± 0.13

[a] MANOVA ns

[b] ANOVA ns

Table 4.2. Numbers of females in "young" and "old" age classes that settled in territories containing surplus cavities versus single cavities. There were no significant differences among treatments ($X^2 = 0.14$, df = 1, $P > 0.6$).

	Relative female age	
Pre-pairing treatment	Young first year return or new breeder	Old adult return (≥ 2 years old)
Surplus cavity territory	22	17
Single cavity territory	19	12

Table 4.3. Numbers of females rearing successful first broods that retained the same territory versus left the territory for the second brood. Females leaving the territory attempted a second brood elsewhere on the study site or left the study site. There were no significant differences among treatments ($X^2 = 2.3$, df = 3, $P > 0.5$).

TREATMENT (pre-pairing/post-laying)	Retained territory	Left territory
Surplus cavities/surplus cavities	7	5
Surplus cavities/single cavity	5	9
Single cavity/single cavity	11	7
Single cavity/surplus cavities	5	5

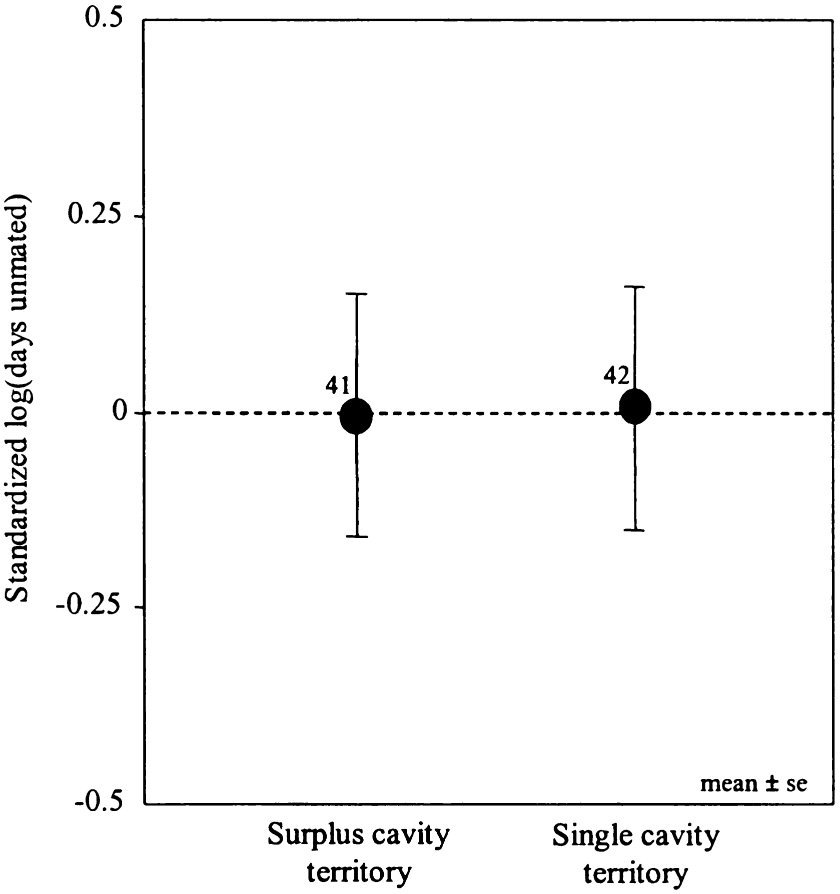

Figure 4.1. Number of days male house wrens with surplus and single cavities in their territories remained unmated (data log transformed and standardized within years) at the start of the breeding season. Means were not significantly different ($P > 0.9$). Sample sizes are indicated.

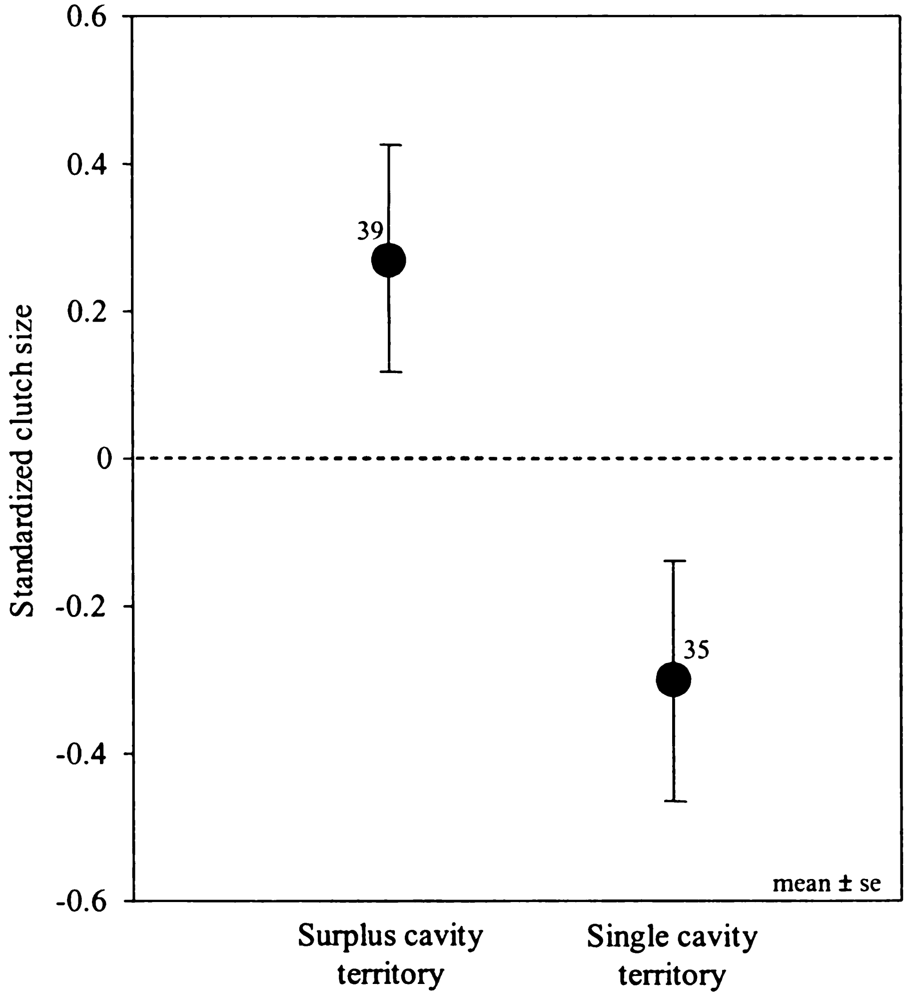

Figure 4.2. The effect of cavity availability on standardized clutch size at early season nests. Means were significantly different ($P < 0.02$). Sample sizes are indicated.

CHAPTER 5

NESTLING SEX RATIOS VARY WITH A MALE TERRITORY CHARACTERISTIC IN HOUSE WRENS

with E. Dale Kennedy and Thomas Getty

ABSTRACT

Sex allocation theory predicts that individuals should bias offspring sex ratios when doing so increases parental fitness. In many cases, females exhibit context-dependent strategies for biasing offspring sex ratios. For example, females paired with attractive mates might bias offspring towards males if attractive males have attractive sons. Females in good condition might bias offspring sex ratio towards sons if males have higher variance in reproductive success. Or, in cases of sex-specific natal philopatry, females might bias offspring sex ratios towards the sex that disperses in order to reduce competition. We manipulated the availability of surplus nest sites, a potential signal of male quality, in male house wren territories. Females mated to males with surplus nest boxes in their territories produced clutches with higher sex ratios (proportion males) than females mated to males with single nest boxes in their territories, although the population offspring sex ratio was not biased. If this facultative adjustment of offspring sex ratios is adaptive, it suggests that the reproductive value of sons and daughters differs for females mated to males with surplus nest sites and females mated to males with single nest sites in their territories.

INTRODUCTION

Fisher (1930) postulated that parents should invest equally in sons and daughters such that, when the costs of producing male and female offspring were equal, offspring sex ratios should be 0.5. Fisher's principle of equal allocation predicts that the optimal offspring sex ratio is the same for all females. Trivers and Willard (1973) argued that selection would favor a conditional strategy of sex ratio allocation when the opportunity costs of investing in sons and daughters vary with parental condition. For example, parents reproducing under favorable conditions might increase lifetime reproductive success by biasing offspring sex ratios towards males when sons in good condition provide higher inclusive fitness benefits to parents than daughters in good condition. The Trivers-Willard hypothesis was framed in terms of polygynous species, where males have higher variance in reproductive success than females, but condition-dependent sex allocation can be applied to any situation in which parents can increase inclusive fitness by biasing offspring sex ratios (Charnov 1982). For birds, offspring sex ratios have been shown to vary with parental condition (Bradbury and Blakey 1998), resource availability (Kilner 1998), and the attractiveness of mates (Sheldon *et al.* 1999).

House wrens (*Troglodytes aedon*) are facultatively polygynous obligate cavity nesters. Offspring sex ratios vary with maternal condition (Whittingham *et al.* 2002), hatch order (Albrecht 2000), and mating status (Albrecht and Johnson 2002), and are biased in the direction predicted by the Trivers-Willard hypothesis. Specifically, these studies have found that females in poor condition or rearing offspring under unfavorable conditions (*e.g.*, secondary females receiving reduced paternal assistance or offspring within the clutch that are at a competitive disadvantage) bias offspring towards females. In previous work (Chapter 4), we found that, during first broods, female house wrens mated to males with surplus cavities in their territories laid larger clutches than females mated to males with single cavities in their territories. We suggested that the observed differences in clutch size might result from female preferences for males able to secure multi-cavity territories (a potential signal of male quality). Females in better condition might have greater access to males with surplus cavities in their territories and favor production of good condition male offspring. Additionally, females mated to attractive males might bias sex ratios towards males if sons inherit attractiveness or viability from their fathers. These scenarios are not mutually exclusive, but in both cases we would expect females mated to males with surplus nest boxes in their territories to produce more male-biased clutches than females mated to males with single cavities in their territories. In this study, we tested the hypothesis that female house wrens bias offspring sex ratios in response to the availability of surplus cavities in their territories.

METHODS

Cavity manipulation

During April through August of 2001 and 2002, we conducted a cavity manipulation experiment on a population of house wrens at the Kellogg Biological Station's Lux Arbor Reserve in Barry County, Michigan (see Chapter 3 for a complete description of the study site and experimental manipulation). We controlled male access to multi-cavity territories by manipulating nest boxes within male territories after male settlement but prior to pairing. Prior to male settlement, all territories contained three nest boxes, but males had access to only a single nest box. The remaining nest boxes had rubber stoppers placed in the entrance holes. After male settlement, but before female settlement, we randomly assigned territories to single or surplus cavity treatments by removing rubber stoppers from the nest boxes in surplus cavity territories (the pre-pairing treatment). Consequently, females chose among males with a single cavity or multiple cavities in their territories at settlement. This treatment was maintained through the pairing and egg-laying periods, at which point we re-randomized treatments across territories (the post-laying treatment, see Chapter 3). We captured breeding females once during the breeding season and marked individuals with a unique combination of color bands. Maternal provisioning rates were observed in each territory on brood days 4, 8, and 12. On brood day 12 we also measured nestling mass, right tarsus length, and right unflattened wing chord.

Offspring sex identification

We collected blood samples from first-brood nestlings on brood day 12. We used a sterile 27G needle to puncture the brachial vein and collected 5-40 μL blood samples in heparinized microcapillary tubes. We then transferred samples to microcentrifuge tubes containing 1 mL of Queen's Lysis Buffer (Seutin *et al.* 1991) and stored samples at 4°C until analysis. During June-October 2003, we extracted DNA from blood samples using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA). We used the PCR-based technique described by Fridolfsson and Ellegren (1999) to determine the sex of nestlings, but modified the annealing temperature specified in the protocol to 46°C or 47°C, depending on the thermalcycler we were using. A single set of primers was used to amplify homologous sections of the CHD-1 gene on the avian sex chromosomes. PCR products were electrophoresed at 90 V on 3% agarose gels for 60-75 minutes and stained with ethidium bromide. When visualized under UV light, males (ZZ) were identified by the presence of a single band approximately 650 bases in size, and females (ZW) were identified by the presence of two bands approximately 650 and 475 bases in size. We sexed 247 nestlings from 43 first brood nests. We validated the sexing procedure by identifying 12 adults of known sex (6 males and 6 females) using this protocol. All were sexed correctly. In addition, we recaptured 14 breeding adults in subsequent years that had been sexed as nestlings. We independently sexed these adults based on behavior and morphological characteristics in the field and then matched them to the results of our molecular sex analysis. Thirteen had been sexed correctly. The identity of the fourteenth could not be determined due to a transcription error in recording keeping.

Statistical analysis

Analyses were conducted in SAS v.8.1 (SAS Institute Inc. 2000). To test whether the experimental treatment affected brood sex ratios, we used generalized linear models (PROC GENMOD) with a logit link function and binomial errors. We used number of male nestlings as the dependent variable and the number of nestlings in the brood as the binomial denominator. We assessed model fit by looking at the residual deviance, which is distributed asymptotically as X^2, and checked standardized residuals for normality. The data were slightly underdispersed (residual mean deviance = 0.67), which leads to conservative tests (Wilson and Hardy 2002). Therefore, we also ran the analysis after rescaling the variance by s (Pearson's X^2 divided by the residual df, Wilson and Hardy 2002). As both methods produced similar results, we report statistics from the latter analysis. Offspring sex ratios are reported as proportion males. Means (± se) are derived from the parameters fitted during statistical analysis. We assessed deviations of treatment means from 0.5 using replicated Goodness-of-Fit tests (Sokal and Rohlf 1995). In order to calculate the G statistic for the Goodness-of-Fit test, we had to exclude one single-box territory nest that contained no males and three females. The excluded nest was an incompletely sexed brood (only three nestlings out of seven eggs survived to BD

12), and its removal had little effect on the average offspring sex ratio for single box territories. Nonparametric tests of clutch size were conducted with Wilcoxon two-sample tests. We sexed offspring from a subset of nests included in the experiment. Therefore sample sizes vary among analyses.

We transformed data where necessary to improve normality and reduce heteroscadiscity of data sets analyzed with parametric tests. We used MANOVAs (PROC GLM) to analyze nestling size data. We compared the average size of male and female nestlings within a nest in order to avoid pseudoreplication, pairing the data by nest in order to control for maternal effects. Because several nests contained only one female or one male, we also analyzed sex differences by comparing sizes for a randomly selected single male and single female nestling from each nest. When MANOVAs were significant we used paired t-tests to examine the individual dependent variables. We excluded one nest from this size analysis that contained only female nestlings.

We analyzed maternal provisioning data (repeated measures) using profile analysis with MANOVA. We included primary and monogamous females, but excluded territories in which another pair of house wrens was able to usurp a ‘surplus’ nest box (this occurred in five territories in 2002, either during the late nestling period of first broods or during second broods). Since clutch sizes varied among treatments (Chapter 4), we looked at both total provisioning rates and per-nestling provisioning rates by females. We were primarily interested in whether any differences in condition between females settling in single box versus surplus box territories might contribute to female provisioning effort and nestling condition. Therefore we included only the pre-pairing treatment as the class variable in these analyses. Means for transformed data are back-transformed into original units.

Females that returned between years were assumed to be independent data points for analysis. Ten females produced first broods during both years, although of these, only five returning females were included in the sex ratio analysis and seven in the provisioning rate analyses. Analyses including returning females are presented, but analyses were also conducted with each female included only once in the data set (randomly selecting which entry to include). These more conservative tests yielded similar results.

RESULTS

Offspring sex ratio

Offspring sex ratio on brood day 12 was significantly higher in territories containing surplus cavities than territories containing single cavities (surplus vs. single, sex ratio = 0.57 ± 0.035 vs. 0.44 ± 0.036; $F_{1,41} = 6.2$, $P < 0.02$; Figure 5.1), but neither offspring sex ratio was significantly different from 0.5 on its own (replicated G-tests: surplus cavities, $G_T = 17.0$, $df = 21$, $P > 0.7$; single cavities, $G_T = 10.5$, $df = 21$, $P > 0.9$). Combining treatments, average brood sex ratios were close to unity in both years (0.50 ± 0.033 in 2001 and 0.52 ± 0.041 in 2002). We were unable to sex unhatched eggs and offspring that did not survive to brood day 12. Sixty percent of nests (26 out of 43) contained at least one unsexed offspring, either due to unhatched eggs or unsexed nestlings. We sexed all of the hatched young in 72% of nests. Neither the occurrence of nests with at least one unhatched egg nor at least one unsexed hatchling differed between treatments (unhatched eggs: $X^2 = 0.18$, $df = 1$, $P > 0.6$; unsexed hatchlings: $X^2 = 2.0$, *df* = 1, $P > 0.15$). On average 1.1 ± 0.26 and 1.0 ± 0.24 offspring (including unhatched eggs) were unsexed in surplus and single cavity territories respectively. When we limited the analysis to nests for which we sexed all hatched young, offspring sex ratios still tended to be higher in territories containing surplus cavities ($F_{1,30} = 3.8$, $P < 0.07$). Of the nests included in the sex ratio analysis, mean clutch sizes were higher in territories with surplus cavities (7.1 ± 0.18 eggs) than in territories with single cavities (6.6 ± 0.12 eggs) but differences were not significant (Wilcoxon two-sample test: $t_s = 1.9$, $P < 0.07$). These nests are a subset of those included in the analyses of clutch size in Chapter 4.

Sex differences in nestling size

Within broods, the average size of male offspring was significantly larger than average size of female offspring (MANOVA controlling for brood effects: $F_{3,39} = 7.8$, $P < 0.001$). Male nestlings had significantly longer tarsi (paired $t = 4.9$, df = 41, $P < 0.0001$) and wings (paired $t = 2.3$, df = 41, $P < 0.03$) and tended to be heavier (paired $t = 1.9$, df = 41, $P < 0.07$) than female nestlings. We obtained similar results when we compared a single randomly selected male and female nestling from each nest (MANOVA controlling for brood effects: $F_{3,39} = 3.6$, $P < 0.03$; tarsus: paired $t = 3.0$, df = 41, $P < 0.01$, wing chord: paired $t = 1.8$, df = 41, $P < 0.09$, mass: paired $t = 2.5$, df = 41, $P < 0.02$; Table 5.1). But these differences were small (males were less than 3.1% larger than females for all morphological variables). We were unable to sex very small runts, and they were excluded from this analysis.

Maternal provisioning effort

We compared provisioning rates of females that settled in single nest box versus surplus nest box territories (excluding the post-laying treatment from our analysis). Total provisioning rates were slightly higher for females that settled in surplus box territories, but differences were not significant (Table 5.2, Figure 5.2). We found no differences in female provisioning rates per nestling between females that settled in single nest box versus surplus nest box territories (Table 5.3, Figure 5.3).

DISCUSSION

As a result of our experimental manipulation, on average, territories in the two treatments should not have differed in male or territory quality, with the exception of cavity availability. Differences in female reproductive investment between the two treatments were likely due to differences in cavity availability. Although we had no reason to believe a priori that the behavior of unmated males would differ between treatments, it is possible that males altered their behavior in response to increased cavity availability, and that female investment might have been a response to male behavior. For instance, males with surplus cavities added to their territories might have increased investment in mate attraction in response to a perceived increase in territory quality. As a result, differences in female reproductive investment could have been an indirect response to cavity availability via male mate attraction effort, rather than a direct response to the cavities themselves. Regardless of the specific signal used by females, we would predict male-biased offspring sex ratios in territories containing surplus nest sites if, under natural conditions, males of higher phenotypic quality were more likely to secure multi-cavity territories and/or females in better condition settled in territories containing surplus cavities.

Under natural conditions, male house wrens compete for cavities and males may physically challenge one another through chases and physical fights (Kendeigh 1941, Johnson 1998). Polygynous house wrens have greater reproductive success than monogamous males (Soukup and Thompson 1998). Since opportunities for polygyny are limited by cavity availability, intense competition among males for access to territories containing multiple nest sites might lead to a correlation between male quality and cavity availability. As a result, cavity availability could indicate male attractiveness or quality. If attractiveness or viability were inherited from fathers, and those characters influence sons more than daughters, then females mated to attractive or high quality males might produce male biased clutches (*e.g.*, Ellegren *et al.* 1996, Svensson and Nillson 1996, Sheldon *et al.* 1999).

Female preferences for high quality males might also lead to females in better condition having greater access to males with surplus nest boxes in their territories, for example if females in better condition return first to the breeding grounds (*e.g.*, Møller 1991). When maternal condition affects offspring condition and has a greater negative effect on the future reproductive success of sons than daughters (such as in polygynous species where males have higher variance in reproductive success), females in good condition should produce male-biased sex ratios relative to those of females in poor condition. In a Wisconsin population of house wrens, females in good condition produced offspring in better condition and had more sons over the breeding season than did females in poor condition (Whittingham *et al.* 2002). The observed male bias in offspring produced by females in good condition was due primarily to male-biased second broods (Whittingham *et al.* 2002). We observed differences in offspring sex ratios during first broods, but we found no differences in the size of females settling in single box and surplus box territories (Chapter 4) or in female provisioning effort between treatments. Nevertheless, we cannot rule out the possibility that the observed relationship between cavity availability and offspring sex ratio might be mediated by differences in female condition between treatments, rather than a direct response to the availability of surplus nest boxes in male territories.

We found that male nestlings were larger in size than female nestlings, although differences were small. If one sex is more costly to produce, then females in poor condition might be restricted to producing the cheaper sex (Myers 1978). While we could not measure sex-specific provisioning rates, females that settled in single box territories did not provision nestlings at lower rates than females that settled in surplus box territories. Other studies have not found evidence for sex-specific provisioning rates (Albrecht and Johnson 2002). These results do not suggest sex-specific differences in costs during the nestling period, although costs could manifest themselves during egg formation if males hatch from larger eggs (Cordero *et al.* 2000) or from eggs containing more androgens (Petrie *et al.* 2001).

Most nests (56%) contained at least one unhatched egg and 28% contained at least one unsexed young (usually due to mortality before brood day 12), but neither the frequency of unhatched eggs or unsexed offspring differed between pre-pairing treatments. Kilner (1998) suggested that food availability might affect the direction of sex-biased mortality in zebra finches (*Taeniopygia guttata*). If house wrens are capable of identifying the sex of nestlings, then selective brood reduction might be possible. In our study, mortality of hatched young was low (less than 5%). Even when we limited our analyses to nests from which all hatchlings were sexed, we observed the same patterns in offspring sex ratio between females mated to males with surplus versus single cavities in their territories. Therefore, it seems unlikely that the observed differences in offspring sex ratios were due to sex-biased mortality. However, we do not know if sex bias occurred at laying or whether it might have resulted from differential hatch success. Since there were no differences in the occurrence of hatch failure between nests in single cavity and surplus cavity territories, hatch failure would have had to be male biased in single box territories and female biased in surplus cavity territories in order to account for the observed differences in offspring sex ratio. If sons are more costly to produce than daughters, male-biased mortality might be more likely in single box territories if those females were in poorer condition and incubated less than females mated to males with surplus cavities. It is more difficult to explain why embryo mortality would be female biased in surplus cavity territories.

Costs of male and female offspring might differ if sex-specific natal philopatry resulted in competition between parents and philopatric young for access to resources (local resource competition: Clark 1978). When natal philopatry differs between the sexes, parents might reduce competition with offspring by producing more of the dispersing sex (Gowaty 1993). Reported rates of natal philopatry are low for house wrens (generally less than 5%) with males returning more frequently than females (Drilling and Thompson 1988). We observe similar return rates on our study site, and approximately 60% of natal returns are males (Dubois, unpublished data). Male-biased offspring sex ratios in surplus nest box territories could be consistent with local resource competition if females residing in territories with surplus nest boxes perceived reduced nest site competition (*i.e.*, there were lots of available nest sites) and invested more in the philopatric sex than females residing in single box territories. However, low return rates, combined with small differences in return rates between male and female offspring make it unlikely that local resource competition would have a large impact on biases in offspring sex ratios.

We have suggested that female preferences for males with territories containing surplus cavities account for the observed differences in offspring sex ratio, either as a result of assortative mating among preferred males (or males in preferred territories) and females in good condition or as a result of differential investment in reproduction by females mated to preferred males. In order for female condition to account for the observed bias in offspring sex ratios, male offspring would need to be significantly more costly to produce than females, or maternal condition would need to have a greater impact on the future reproductive success of sons than daughters. Alternatively, if sons of males with high polygyny potential are more likely to be polygynous themselves, females mated to attractive mates might bias offspring towards males. Research on the heritability of traits associated with male polygyny potential (or some other indirect benefit inherited by sons), and whether cavity availability reliably signals male quality in natural situations, would be needed to confirm this hypothesis.

In summary, we found that female house wrens mated to males with surplus cavities in their territories produced clutches with higher offspring sex ratios than females mated to males with single cavities in their territories. The observed sex ratio bias was likely due to differences in the sex ratio at laying rather than differential mortality during the nestling period. Our results are consistent with predictions of the Trivers-Willard (1973) hypothesis based on differences in reproductive value of offspring under different breeding conditions. Our results may thus have interesting implications for nest box studies, especially those utilizing high densities of nest boxes for polygynous species. Close spacing of nest boxes probably increases the occurrence of multi-cavity territories compared to natural cavities and might have consequences on female reproductive investment, including the sex ratio of offspring produced.

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Table 5.1. Size of male and female offspring on brood day 12.[a]

MORPHOLOGICAL VARIABLE	Male offspring	Female offspring
Mass (g)	10.5 ± 0.10	10.2 ± 0.12
Tarsus (mm)	19.5 ± 0.08	19.2 ± 0.09
Wing (mm)	34.5 ± 0.30	33.9 ± 0.38

[a] Analysis comparing a randomly selected male and female nestling from each nest. MANOVA significant at the $P < 0.03$ level. See text for alternate method of analysis.

Table 5.2. Repeated measures analysis of total maternal provisioning rates on brood days 4, 8, and 12 for females that settled in territories containing surplus nest boxes and single nest boxes (pre-pairing treatment) at early season nests.[a]

A. MANOVA (profile analysis) of within subject effects. Pillai's trace values are presented.

Source	Num df	Den df	Value	*F*	*P* > *F*
Brood day (time)	2	54	0.67	54.1	0.0001
Brood day × Treatment	2	54	0.01	0.27	0.7

B. Repeated measures ANOVA of between-subject effects.

Source	df	MS	*F*	*P* > *F*
Treatment	1	2.5	1.7	0.19
Error	55	1.5		

C. ANOVAs on each of the contrasts for time differences between successive observations. Only the brood day effect is shown, others were non-significant.

Source	df	MS	*F*	*P* > *F*[b]
Contrast variable: BD8 – BD4				
Brood day	1	56.3	69.8	0.0001
Error	55	0.8		
Contrast variable: BD12 – BD8				
Brood day	1	5.3	4.6	0.04
Error	55	1.1		

[a] Data square root transformed. Nests with incomplete data were excluded.

[b] Bonferroni adjusted $\alpha = 0.025$.

Table 5.3. Repeated measures analysis of maternal provisioning rates per nestling on brood days 4, 8, and 12 for females that settled in territories containing surplus nest boxes and single nest boxes (pre-pairing treatment) at early season nests.[a]

A. MANOVA (profile analysis) of within subject effects. Pillai's trace values are presented.

Source	Num df	Den df	Value	*F*	*P* > *F*
Brood day (time)	2	54	0.67	55.1	0.0001
Brood day × Treatment	2	54	0.03	1.0	0.3

B. Repeated measures ANOVA of between-subject effects.

Source	df	MS	*F*	*P* > *F*
Treatment	1	0.03	0.48	0.4
Error	55	0.06		

C. ANOVAs on each of the contrasts for time differences between successive observations. Only the brood day effect is shown, others were non-significant.

Source	df	MS	*F*	*P* > *F*[b]
Contrast variable: BD8 – BD4				
Brood day	1	2.3	78.1	0.0001
Error	55	0.03		
Contrast variable: BD12 – BD8				
Brood day	1	0.15	3.7	0.06
Error	55	0.04		

[a] Data reciprocal square root transformed. Nests with incomplete data were excluded.

[b] Bonferroni adjusted $\alpha = 0.025$.

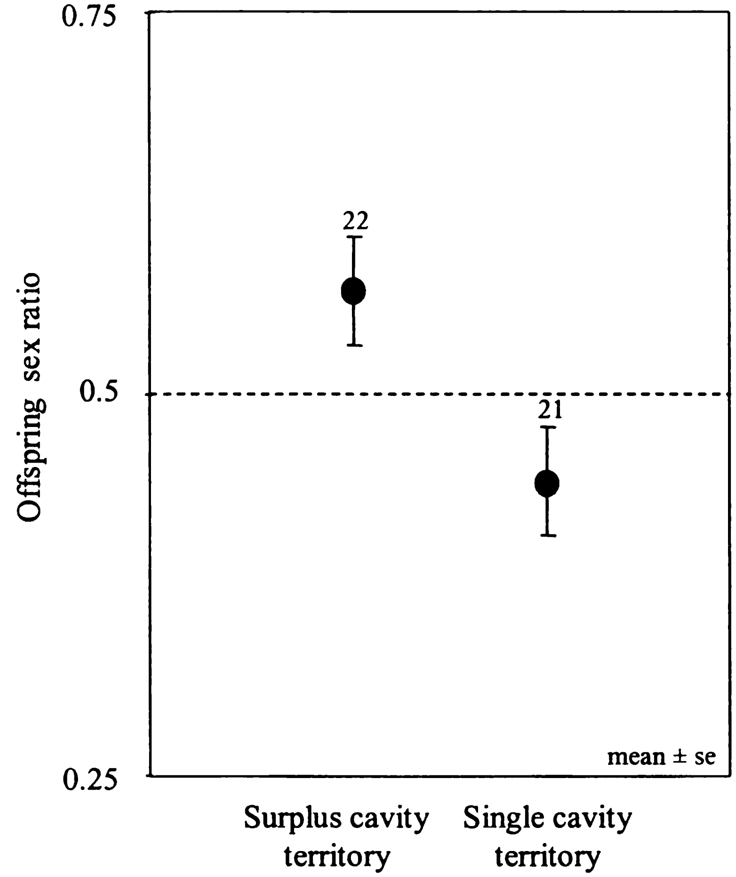

Figure 5.1. Average offspring sex ratios (proportion males) of clutches produced by females mated to males with surplus and single cavities in their territories. Means were significantly different at the $P < 0.02$ level. Sample sizes are indicated above the symbols.

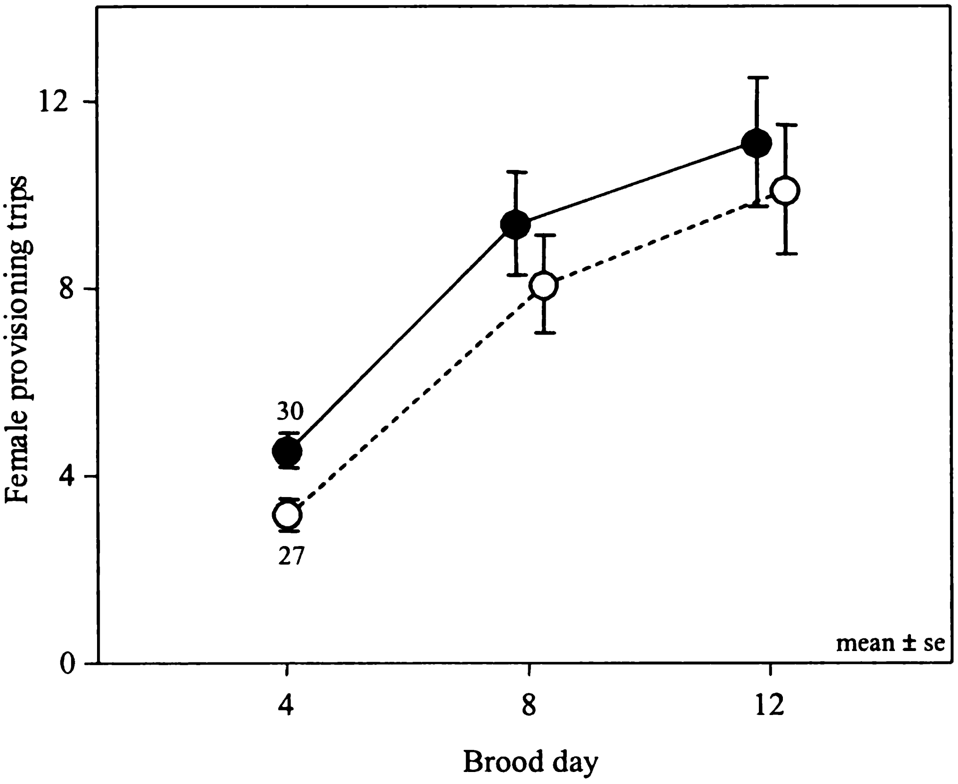

Figure 5.2. Average total maternal nestling provisioning rates during 30-minute observation periods for females that settled in territories containing surplus nest boxes (solid symbols) and single nest boxes (open symbols) through the laying period. Only the time effect was significant ($P < 0.0001$). Sample sizes are indicated.

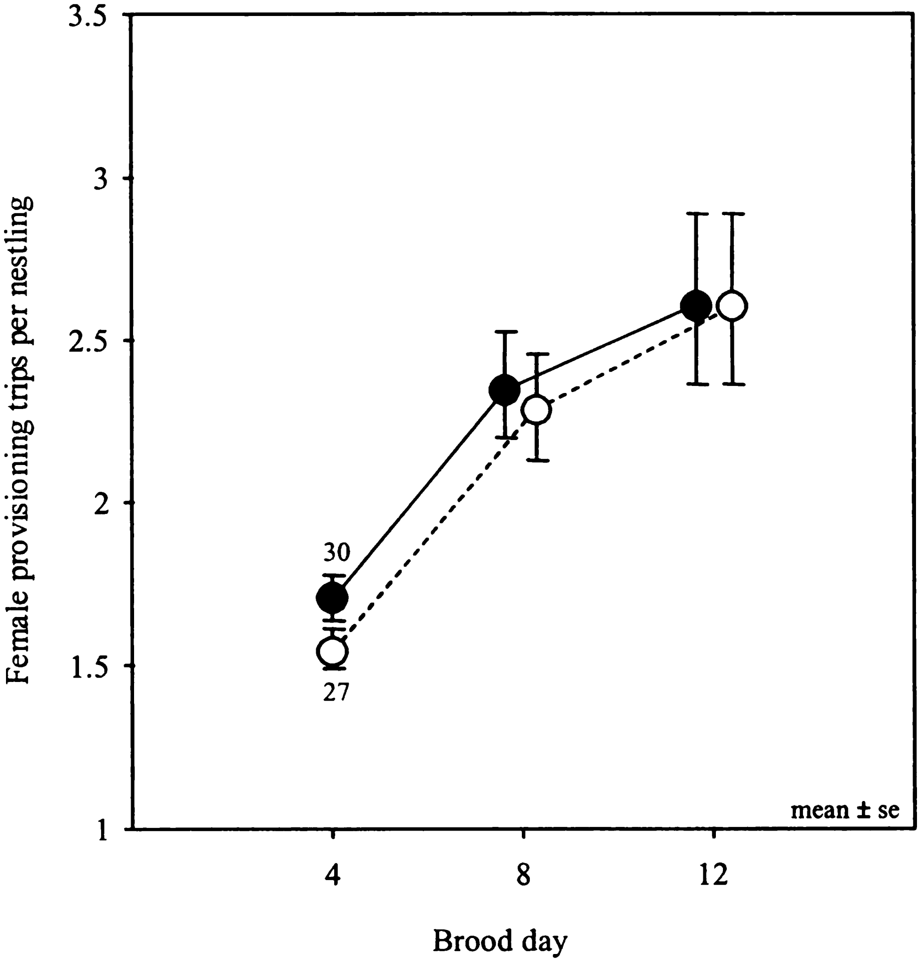

Figure 5.3. Average maternal provisioning rates per nestling during 30-minute observation periods for females that settled in territories containing surplus nest boxes (solid symbols) and single nest boxes (open symbols) through the laying period. Only the time effect was significant (*P* < 0.0001). Sample sizes are indicated.