This is to certify that the
thesis entitled

MATE QUALITY AND PARENTAL INVESTMENT IN THE
HOUSE WREN

presented by

Lindsey A. Walters

has been accepted towards fulfillment
of the requirements for the

Ph.D. degree in Zoology and Ecology, Evolutionary Biology, and Behavior

Thomas Getty
Major Professor's Signature

2 December 08
Date

MATE QUALITY AND PARENTAL INVESTMENT IN THE HOUSE WREN

By

Lindsey A. Walters

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOHPY

Zoology
Ecology, Evolutionary Biology, and Behavior

2008

ABSTRACT

MATE QUALITY AND PARENTAL INVESTMENT IN THE HOUSE WREN

By

Lindsey A. Walters

Iteroparous organisms face a fundamental life history tradeoff between investment in current and future reproduction. Theory suggests that organisms should bias their reproductive investment based on the marginal fitness returns for each breeding attempt. A high quality mate can improve the return on investment either directly, by providing better parental care and resources, or indirectly, by providing genetic benefits to the offspring. Additionally, organisms can increase their reproductive success by increasing their parental investment when they have an attractive mate, an idea called differential allocation. In this dissertation, I investigated how mate quality influences parental investment in the facultatively polygynous house wren (*Troglodytes aedon*).

House wren males defend territories containing cavities for females to nest in and also assist their mates in provisioning the nestlings. I investigated how male territory quality, a potential indicator of male quality, influenced provisioning by both sexes. I manipulated male territories by randomly providing some males with multiple cavities on their territories after female settlement, giving them the opportunity to attract additional mates. I found that males that were randomly assigned multiple cavities provisioned their nestlings at significantly lower rates than males randomly assigned one cavity, which may be the result of a tradeoff between male parental care and mate attraction. However, in contrast to earlier studies, we found no evidence for female differential allocation of maternal investment.

I also investigated a potential mechanism for male differential allocation based on female quality. Recent work suggests that avian egg color could be a sexually selected signal to males of female quality and/or of costly female investments in the egg. If female quality or investment is high, this would mean that a male's own investment in the offspring would generally yield high fitness returns. I performed a cross fostering experiment to test whether egg color was related to female condition or maternal investment and whether male provisioning of nestlings was related to egg color. I found that brighter eggs (i.e. those with less brown pigment) were heavier, and that nestlings that hatched from brighter eggs were fed at higher rates by their foster mothers but not their foster fathers. These results suggest that females who lay brighter eggs also invest more in those offspring through maternal effects on egg content, although males do not seem to differentially allocate their investment based on this potential cue.

Finally, in a study spanning six years, I observed that female differential allocation of clutch size based on the manipulated number of cavities in male territories was not consistent from year to year. The difference in clutch size between females mated to high versus low quality males declined systematically over time. I tested whether this linear decline was related to other factors that also might vary systematically with time such as weather, female age, and population density. The differential female response was strongly negatively correlated with local wren density, suggesting that differential allocation may be sensitive to population density. More research is needed to understand how and when mate quality affects parental investment.

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the help and support of many people. I would like to thank my advisor, Tom Getty, for his guidance, encouragement, friendship, and good humor. He has helped me become a better scientist. The rest of my guidance committee, Fred Dyer, Kay Holekamp, and Andrew McAdam, were also essential in completing this dissertation, providing valuable suggestions about research ideas through the years and giving insightful comments on manuscripts.

I also received tremendous support from my labmates, the "Getty Girls". Katie Wharton was my rock and closest friend as we progressed through the ups and downs of graduate school together, and I can never thank her enough. Natalie Dubois was instrumental in starting the house wren project, and I am very grateful that she left me with such a smoothly functioning system to work with. Eva Lewandowski and Jean Johnson also contributed to this project through advice, encouragement, and friendship.

I have been lucky enough to have an amazing field assistant, Brian Maitner, who worked on the wren project for three years and never complained once about the prevalent bugs, heat, thorns, or wren droppings. Jean Johnson, Rachel Mitchell, Chris Keller, and Lydia Roach also provided crucial help in the field and lab. I am also grateful to the many friends and family members who helped put up nest boxes every year, to Kimberly Sheldon and Stefan Lüpold for teaching me how to work with birds, and to Joe Johnson and Karen Charleston for allowing me to work under their banding permits.

Finally, I would like to sincerely thank my friends and family, and especially my parents, for their tremendous emotional, mental, and spiritual support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Iteroparous organisms face a tradeoff between investment in current and future reproduction (Stearns 1989). Parental investment by an organism in its current breeding attempt by definition reduces its future reproductive success (Trivers 1972; Clutton-Brock 1991). Therefore, organisms face the challenge of deciding how best to allocate their resources toward each reproductive attempt in a way that will maximize their lifetime reproductive success.

Both males and females can increase their reproductive success by mating with a high quality mate. Historically, this idea has been studied from the perspective of females that benefit by choosing to mate with a high quality male. In this scenario, high quality males provide direct or indirect benefits to females (Moller and Thornhill 1998; Kokko et al. 2003). High quality males can directly provide benefits like better parental care or more resources. For example, male red winged blackbirds with bills that are more orange, a signal of male quality, also provide more parental care (Preault et al. 2005). High quality males can also indirectly provide benefits like better genes or more viable offspring. For example, Rundle et al. (2007) found that male fruit flies descended from more attractive males had higher mating success than those descended from less attractive males. Despite the common focus on the benefits to females of male quality, males can also benefit from mating with a high quality female (Amundsen 2000). For example, male lagoon gobies prefer to mate with females who have larger belly patches, and males benefit from the preference because these females lay more eggs (Pizzolon et al. 2008).

Individuals that manage to secure a high quality mate might have to pay a price in reproductive investment (Moller and Thornhill 1998), an idea called differential allocation (Burley 1986). If the marginal fitness returns on investments in offspring vary across breeding attempts and are higher during breeding attempts with high quality mates, then individuals should increase their investment when they happen to breed with a high quality mate (Burley 1986; Burley 1988; Sheldon 2000). These higher quality mates in turn should have greater lifetime reproductive success because they can decrease their parental investment due to this compensation by their partner (Burley 1986). Differential allocation theory provides a useful framework that biologists can use to make predictions about the fitness consequences of parental investment for both partners when mate quality varies.

One specific type of parental investment that can be differentially allocated is parental care of young. In species where both parents provide parental care, such as most birds (Ketterson and Nolan 2004), males and females typically experience asymmetries in the optimal amount of parental care they should provide for each breeding attempt. In these species, sexual conflict occurs because the costs of investment in parental care are paid separately by each parent but both parents reap the fitness benefits. Therefore, both sexes would benefit if the other sex worked harder so they could reduce their costly investment in parental care (Chapman et al. 2003; Wedell et al. 2006). In addition, males typically have the potential to increase their reproductive success by sacrificing investment in parental care in order to spend time attracting additional mating opportunities (Magrath and Komdeur 2003). Females may counter by soliciting more extra pair copulations during future reproductive bouts when their social mate is a poor provisioner (Freeman-Gallant 1996). These types of sexual conflict are likely to have been an important factor influencing the evolution of parental investment and sexual selection (Trivers 1972; Chapman et al. 2003; Olson et al. 2008).

Although differential allocation of parental investment is gaining recognition as an important component of sexual selection (Sheldon 2000; Head et al. 2006), empirical tests of differential allocation have yielded mixed results. Many studies have indeed found that mate quality positively influences parental investment (e.g. Osorno et al. 2006; Roed et al. 2007; Skinner and Watt 2007). For example, peahens randomly mated to males with more tail ornaments, a signal of male quality, increased their parental investment in eggs by laying heavier eggs and depositing more testosterone in the egg yolk (Loyau et al. 2007). However, many other studies testing for differential allocation have yielded negative or inconsistent results (e.g. Cooper and Ritchison 2005; Hargitai et al. 2006; Ferree 2007, Nakagawa et al. 2007). More research is needed to understand how and when mate quality influences differential allocation of parental investment. This dissertation considers situations where differential allocation is expected but either has not been tested for, or has yielded inconsistent results, in order to help fill in the gaps about the when differential allocation should be favored by natural selection. Specifically, I examine the interplay between differential allocation and sexual conflict over parental care, rarely-studied male differential allocation in response to female quality, and the impact of demographic factors such as population density on differential allocation.

In this dissertation, I examine how mate quality affects parental investment in the facultatively polygynous house wren (*Troglodytes aedon*). House wrens are an ideal species for studying this question. First, both male and female house wrens provide parental investment by provisioning the nestlings, and male parental care is especially important for nestling survival early after hatching while females are still brooding the nestlings (Bart and Tornes 1989; Johnson et al. 1992). This bi-parental care leads to the potential for sexual conflict between males and females over the amount of parental care they each provide. Another advantage to studying differential allocation in this species is that males defend territories that contain cavities for females to nest in, and researchers can conveniently manipulate male territory quality by manipulating the number of available cavities on male territories after female settlement.

In chapter 2, I use a manipulation of the number of nest boxes on a male's territory to examine how male territory quality influences female differential allocation in the house wren. The number of available cavities on a male's territory would normally be related to male competitive ability and has been shown in other studies to influence female investment (Eckerle and Thompson 2006; Dubois et al. 2006). I explore whether males randomly assigned multiple nest boxes reduced their investment in provisioning compared to males with one nest box. This could occur either because of the increased opportunities for mate attraction available to them or because of increased compensation by their partners due to differential allocation, which would be a form of counter-compensation by males.

In chapter 3, I examine differential allocation from the male perspective. Recent work suggests that avian egg color could be a sexually selected signal to males of female condition or genetic quality and/or of costly female investments in the egg (Moreno and Osorno 2003). If female quality or investment is high, this would mean that a male's own investment in the offspring would generally yield high fitness returns. The primary pigment in blue eggshells, biliverdin, is a potentially biologically costly antioxidant (McGraw 2005), and some studies have found that males increase their parental care when they have more intensely blue eggs in their nest (Moreno et al. 2006b, Soler et al. 2008). However, no one has addressed whether egg color influences male provisioning in species of birds like the house wren that lay brown eggs, where the primary pigment in eggshells, protoporphyrin, is a pro-oxidant associated with poor female condition (Gosler et al. 2005; Martinez-de la Puente et al. 2007) . I present the results of a cross fostering experiment with house wren eggs that tested whether egg color was related to female condition or maternal investment in the eggs and whether male provisioning of chicks was related to egg color.

In chapter 4, I examine variation across years in female differential allocation. In this study spanning six years, female response to male quality varied from year to year. Early on, females mated to a male with one nest box in his territory had significantly smaller clutches than females mated to a male with three nest boxes in his territory. However, the difference in clutch size between females in the two treatments declined over time. I present results from an analysis looking for factors that might influence differential allocation and that also might change systematically over time, including weather, female experience, and population density. I determine whether any of these factors might help to explain the diminishing differential allocation observed in this population.

CHAPTER 2
MALE HOUSE WRENS WITH THE OPPORTUNITY TO ATTRACT ADDITIONAL MATES PROVIDE LESS PARENTAL CARE

with Thomas Getty

Abstract

Sexual conflict over parental care can lead to asymmetries in parental investment between males and females. In many species, males can benefit by sacrificing parental care in order to pursue additional mates, and females can benefit by providing greater parental investment when they are mated to a more attractive male. We studied these tradeoffs in the facultatively polygynous house wren (*Troglodytes aedon*). We randomly provided some males multiple nest boxes on their territories, giving them the opportunity to attract additional mates. Earlier work showed that this can also increase maternal investment by the original mate. We found that males that were randomly assigned three nest boxes provisioned their nestlings at lower rates early after hatching than males that were randomly assigned one nest box and at higher rates late after hatching. However, in contrast to earlier studies of this population, we found no evidence for female differential allocation of maternal investment. There were also no differences between males with multiple versus single nest boxes in retaining their territories for an additional breeding attempt. Our results highlight the need for more research into the role of this tradeoff in influencing sexual conflict over parental care.

Introduction

Parental care is an important but costly class of behaviors that affect fitness in many species (Trivers 1972; Clutton-Brock 1991). In species with biparental care, sexual conflict can occur over the amount of parental care provided by each parent (Houston et. al 2005; Wedell et al. 2006; Olson et al. 2008). This conflict plays out differently for each sex. Typically, males have the potential to increase their reproductive success by increasing their efforts to obtain extra-pair copulations or additional mates, behaviors that can come at the expense of parental care (Magrath and Komdeur 2003). In contrast, females can typically increase their lifetime reproductive success either directly, by choosing to mate with males who will provide more parental care, or indirectly, by mating with attractive males who will sire attractive or healthy sons and daughters (Kokko 1998; Moller and Thornhill 1998).

When females have the opportunity to mate with a preferred male, they can sometimes further increase their lifetime fitness by increasing their investment in parental care, an idea called differential allocation (Burley 1986). Increased allocation is expected when the marginal fitness returns on parental investments are higher during breeding attempts with more attractive mates. The logic behind this idea is that there is a synergy between male and female contributions that results in accelerating fitness returns to females. However, the nature of this tradeoff between current and future reproductive success will also be influenced by an organism's probability of future reproductive success (Sheldon 2000). For example, an individual that is not likely to breed again should invest maximally in its current reproductive attempt regardless of the attractiveness of its mate because the chances of finding a more attractive partner in the future are low. The differential allocation hypothesis further predicts that if the mates of attractive males increase their parental investment, these attractive males should be able to decrease their current parental investment to save more investment for future breeding attempts, assuming that the value of parental investment for an individual is constant across years (Burley 1986). Therefore, male attractiveness has the potential to influence the amount of investment in parental care provided by both females and males.

Biparental care is common in birds (Ketterson and Nolan 1994), and many studies have found that preferred males provide less parental care (e. g. Burley 1988; deLope and Moller 1993; Qvarnstrom 1997; Duckworth et al 2003), suggesting that male attractiveness results from indirect genetic benefits in these situations. When this is the case, attractive males might be able to decrease their provisioning efforts because of compensation by their partners (Magrath and Komdeur 2003). This opportunity to spend less time provisioning could be especially valuable to males because it would allow them to spend more time and energy trying to attract additional mates.

We used the house wren (*Troglodytes aedon*), a facultatively polygynous, double-brooded, secondary cavity nester (Johnson 1998), to examine the effects of male attractiveness on both female and male investment in parental care. We manipulated the number of cavities in a male's territory to manipulate male attractiveness. Because of the intense competition between males for territories (Kendeigh 1941; Johnson 1998), we hypothesized that a male's territory reflects his quality and therefore his attractiveness, i.e. his ability to provide fitness benefits to his offspring. Previous studies have shown that female house wrens preferred to mate with males who had multiple nest boxes on their territories (Eckerle and Thompson 2006), and that females mated to males with multiple nest boxes differentially allocated their parental investment by laying larger and more male-biased clutches (Dubois et al. 2006).

For our study, all males had one nest box available on their territory until after females had settled. Then, we randomly assigned half of the males in our population to have two additional nest boxes, with the remaining males continuing to have one nest box. We also cross-fostered nestlings to control for pre-incubation maternal effects. Male house wrens who randomly receive a territory with multiple cavities could have two possible reasons to decrease their investment in parental care. First, if multiple cavities signal to females that a male will provide higher fitness benefits for her offspring, she might differentially allocate her parental care and increase her provisioning efforts, allowing the male to counter-compensate and decrease his investment in provisioning. Second, multiple cavities give a male the opportunity to increase his fitness by attracting a second mate (Soukup and Thompson 1998), providing further incentive for him to decrease his parental care and spend more time and energy on mate attraction. Therefore, we predicted that the males with three nest boxes would reduce their investment in provisioning compared to males with one nest box. In house wrens, male provisioning of nestlings seems to be especially important early after hatching, when females are still brooding the nestlings (Bart and Tornes 1989; Johnson et al. 1992), so we were most interested in testing whether preferred males decreased their provisioning during this crucial time.

Methods

We conducted our study in the summer of 2007 using a wild population of house wrens at Lux Arbor Reserve in southwest Michigan. Prior to wren arrival in the spring, we established 53 territories that each contained three nest boxes. This study site has few natural cavities, and there were few, if any, wrens breeding in the area that were not nesting in one of our boxes. Boxes within a territory were spaced within 15 m of each other, and territories were spaced approximately 100 m apart so that male wrens could only defend boxes on one territory at a time. Two of the boxes in each territory were plugged using rubber stoppers to prevent access to the nest box, so only a single nest box was available to all the wrens when they arrived. We checked boxes at least every other day and recorded status of the nest and any adults observed defending the nest or singing.

After both a male and female had arrived on a territory, we randomly assigned the pair to either the one box or three box treatment. Assignments where paired to control for establishment date. For territories in the three box treatment, we removed the rubber stoppers from the two extra nest boxes, providing the breeding pair with access to three boxes. We considered a territory to be occupied by a breeding pair once the cup nest was lined. Although both males and females build the stick portion of the nest (Alworth and Scheiber 2000), this lining behavior is uniquely performed by the female (Kendeigh 1952). By waiting until after female arrival to apply the treatment, we eliminated the possibility of differential female access to attractive three box males and any confounding of female quality with territory treatment.

In order to control for any potential genetic or maternal effects on nestling begging behavior, we cross fostered entire clutches of eggs between nests so that parents were not provisioning their genetic offspring. We performed this manipulation on 50 different nests in the early part of the breeding season, before any wrens began a second breeding attempt. We swapped 50 entire clutches of eggs (25 swaps) between nests that had completed laying on the same day (20 swaps) or within one day of each other (5 swaps). We performed this swap the day after clutch completion (i.e. the first day that we found no new egg in the nest). We also paired swapped clutches so that the clutch size of both nests was the same (16 swaps) or differed only by one egg (8 swaps). One swap was made between nests whose clutch size differed by 3 because they were the only clutches that were completed at that time. Swaps were made randomly in regard to treatment, so some swaps occurred between treatments and some occurred within treatments.

We captured and measured adults prior to nestling hatching and gave them a unique combination of color leg bands so that we could recognize individuals without recapturing them. In order to determine hatching date, we checked boxes every day starting at ten days after the clutch was completed. We measured parental provisioning rates two times after hatching: early (day 4 to 6 after hatching) and late (day 10 to 11 after hatching). We were particularly interested in differences between treatments at the crucial early feeding observation, but we conducted the late feeding observation to assess whether provisioning rates changed over time as the nestlings grew older and females where able to spend less time brooding and more time foraging and provisioning the nestlings. For each feeding observation, we observed nest boxes from a distance using binoculars for 30 minutes and recorded all visits made by both parents. If the parents were disturbed by our approach, we waited until after they had resumed normal feeding to begin our observations. We used the total number of visits per nestling per 30 minutes for each parent as a standardized measure of provisioning effort in our analyses. At ten days after hatching, we banded each nestling and measured their mass.

Four of the fifty nests were destroyed by predation before hatching, and one was destroyed between the first and second provisioning observation, so we do not have provisioning data for these nests. We also did not include provisioning rates from two nests where we could not conclusively differentiate between the male and female during the provisioning observations because we could not find an unobstructed view of the nest box. Only one instance of polygyny occurred during this study, and this nest was also excluded from our analyses. This low rate of polygyny was consistent with previous studies at this site (Dubois 2004; Dubois et al 2006); it probably results from female defense of the closely spaced empty boxes (Slagsvold et al. 1999).

After nestlings had fledged or the nest was destroyed by predation, we removed the used nesting material from the nest box. If the territory had been in the three box treatment, we replaced the corks in the two auxiliary boxes so that all territories again had only one open nest box for the second brood. We continued to monitor boxes at least every other day through the end of the breeding season. If the box was used again for a second brood, we established the identity of the parents either through observation of band colors or by recapturing adults for whom we were unable to make a visual identification.

To test for the effects of treatment on provisioning rates of males and females, we used mixed effects general linear models. Our response variable was the provisioning rate of either males or females, and our predictor variables were observation time (early or late), treatment (1 box or 3 box), and the interaction between observation time and treatment. We included individual bird as a random factor to account for repeated measures of individual birds at the early and late observations.

We also tested to see if male and female provisioning rates were correlated at each provisioning observation. We performed a correlational analysis using the non-parametric Sprearman's rank correlation coefficient (ρ) because of heteroscedasticity in the data at the late provisioning observation. We used chi-squared tests to determine if treatment influenced whether an individual would breed twice in one summer. We performed all statistical analyses using the program R (R Core Development Team, 2006).

Results

Male and female provisioning rates responded differently to treatment and the time of the observation (Figure 2.1). Contrary to our predictions, female provisioning rates did not differ between treatments ($F_{1,40}$=0.013, p=0.910), but across treatments females provisioned significantly more at the second provisioning observation than at the first ($F_{1,40}$=18.7, p<0.001). For the females, there was no interaction between treatment and the time of the observation ($F_{1,40}$=0.465, p=0.490). However, for male provisioning rates, there was a significant interaction between treatment and time ($F_{1,40}$=10.2, p=0.003). At the crucial early provisioning observation, three-box males provisioned less than one-box males (1 box: 1.26, s.e.=0.20; 3 box: 0.72, s.e.=0.13), consistent with our predictions. In contrast, at the later provisioning observation, three box males

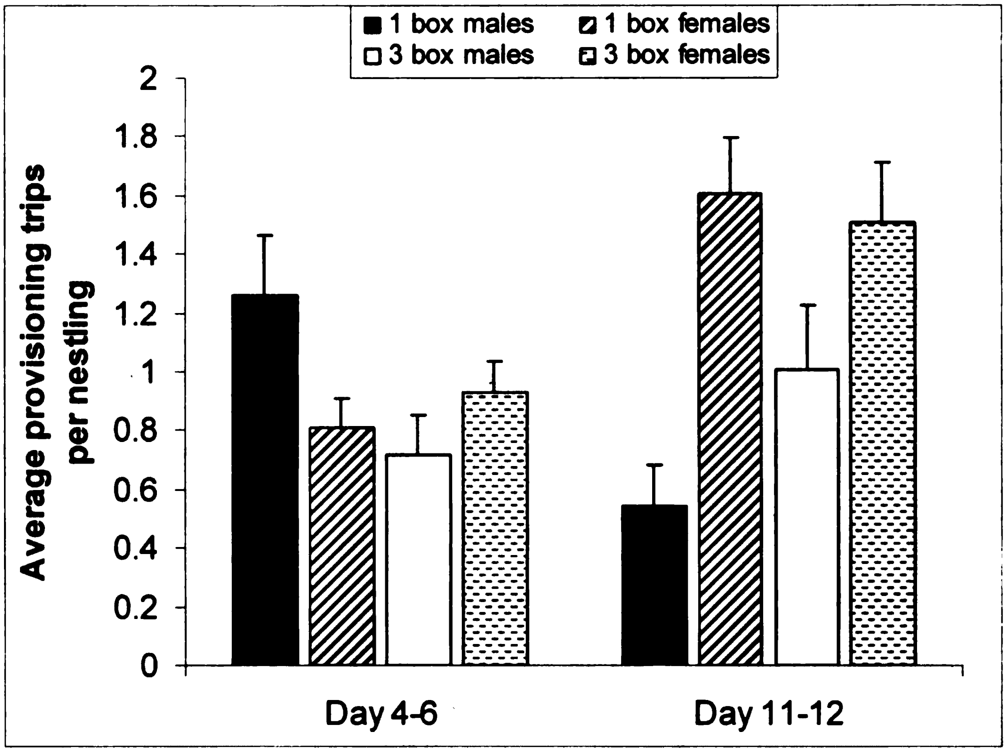

Figure 2.1. Average provisioning trips per nestling during a 30 minute observation early (day 4-6) and late (day 11-12) after hatching for males with one nest box (filled bar), females mated to males with one nest box (diagonal stripes), males with three nest boxes (open bar) and females mated to males with three nest box (horizontal dashes). Treatment did not predict female provisioning rates ($F_{1,40}$=0.013, p=0.910), but across treatments females provisioned more at the late observation than at the early observation ($F_{1,40}$=18.7, p<0.001). Treatment and time of provisioning observation interacted significantly to predict male provisioning rate ($F_{1,40}$=10.2, p=0.003). Males with one nest box provisioned at higher rates than males with three nest boxes at the early observation and at lower rates at the late observation.

provisioned at higher rates than one box males (1 box: 0.54, s.e.=0.15; 3 box: 1.00, s.e.=0.22). Neither treatment ($F_{1,40}$=0.0619, p=0.8047) nor time of the observation ($F_{1,40}$=1.96, p=0.169) had any additional effect on male provisioning rates. Mean clutch size did not differ significantly between treatments (1 box: 6.77, s.e.=0.20; three box: 6.81, s.e.=0.15; $F_{1,41}$=0.22, p=0.883).

At the early provisioning observation (day 4-6), male and female provisioning rates were not related (S=13380, df=41, p=0.948, ρ=-0.010; Figure 2.2). However, at the later feeding observation, male and female provisioning rates were negatively correlated (S=18150, df=40, p=0.002, ρ=-0.471; Figure 2.3). In addition, treatment did not seem to influence the relationship between male and female provisioning rate. Additional correlational analyses performed separately within each treatment revealed that at the early observation, male and female provisioning rates were not correlated for either treatment (1 box: S=1838, df=41, p=0.868, ρ=-0.038; 3 box: S=1429, df=41, p=0.756, ρ=0.072). At the late observation, male and female provisioning rates were significantly negatively correlated for both treatments (1 box: S=2239, df=40, p=0.039, ρ=-0.454, 3 box: S=2462, df=40, p=0.004, ρ=-0.598).

For both the early and late observations, some parents did not provision at all during the 30 minute time period (Figure 2.2; Figure 2.3, some data points overlap). At the early observation, 7 males did not provision. At the late observation, 16 males and 1 female did not provision. However, we are confident that most of these birds had not abandoned the nest. For all but 2 of the individuals who did not provision, we were able to confirm that they were still present because we observed the bird either singing or alarm-calling at that nest sometime after the observation at which it did not provision.

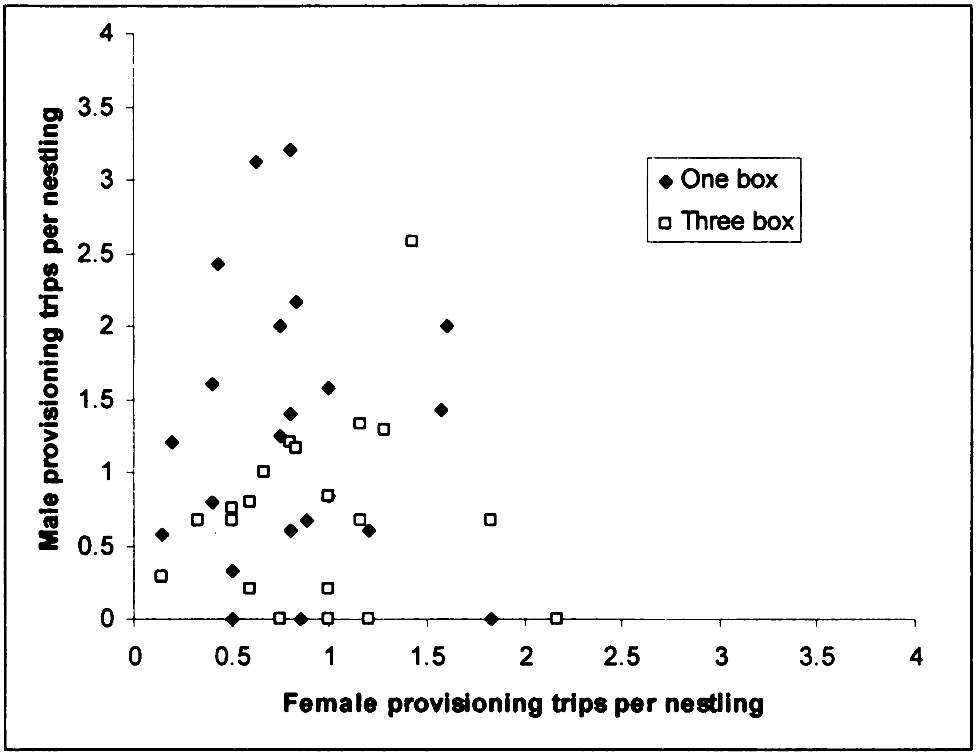

Figure 2.2. Relationship between the number of male and female provisioning trips per nestling for each nest during the 30 minute observation period early (day 4-6) after hatching for pairs in the one box (solid diamonds) and three box (open squares) treatments. The provisioning rates of males and females were not correlated with each other (S=13380, df=41, p=0.948, ρ=-0.010).

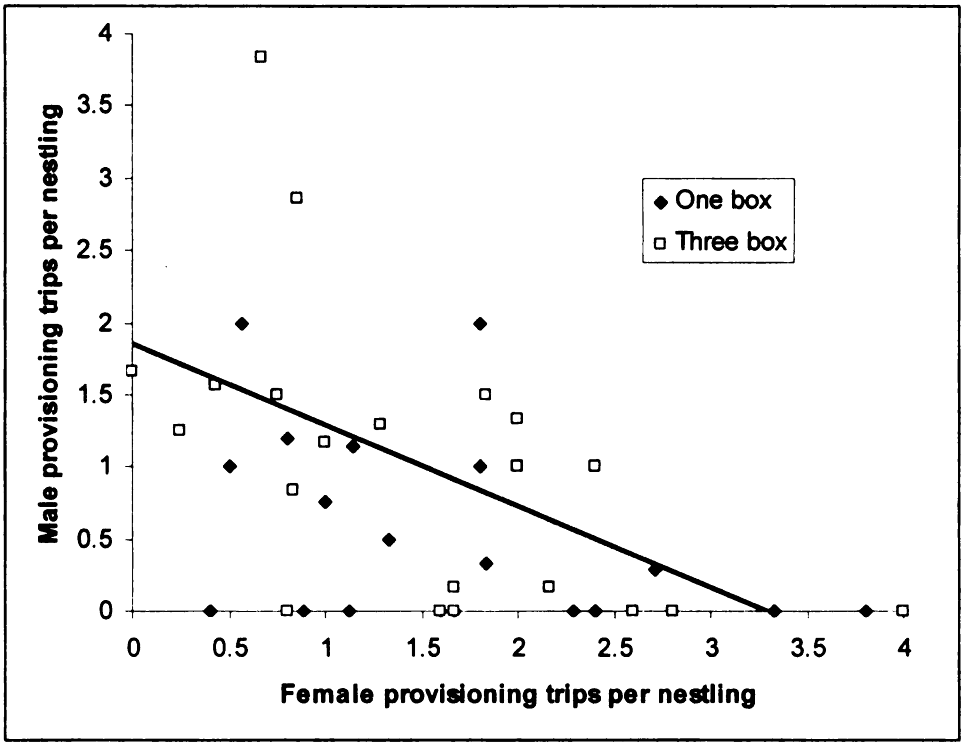

Figure 2.3. Relationship between the number of male and female provisioning trips per nestling for each nest during the 30 minute observation period late (day 10-11) after hatching for pairs in the one box (solid diamonds) and three box (open squares) treatments. The provisioning rates of males and females were significantly negatively correlated with each other (S=18150, df=40, $p=0.002$, $\rho=-0.471$).

The 2 non-provisioning individuals that we could not confirm were still present were both in the one box treatment group. Removal of these individuals from our analyses did not affect the significance of our results. All but 3 of the nests that had a parent who did not provision still managed to fledge all of their nestlings.

The differences between treatments in male provisioning rates at first brood nests did not translate into differences in mean number of offspring fledged from the first brood (1 box: 5.45; 3 box: 5.52, $F_{1,41}=0.022$, $p=0.882$). Overall, male provisioning rate at the first provisioning observation was not related to mean nestling mass for a nest ($F_{1,40}=0.352$, $p=0.556$) or to number of offspring fledged ($F_{1,40}=2.11$, $p=0.154$) in this study. Nestling mass at day 10 was also not related to the overall provisioning rate made by both parents at either observation (early: $F_{1,40}=0.034$, $p=0.856$; late: $F_{1,40}=2.09$, $p=0.156$), despite the large amount of variation in total provisioning visits received by each nest.

Treatment did not affect male success at breeding twice during one breeding season ($\chi^2=0.105$, df=3, $p=0.991$; Table 2.1). Across treatments, males who bred twice almost always did so by holding on to their original territories. Of the 19 males that were able to breed twice in one summer, 18 did so by keeping their original territory. Only 1 male that lost his original territory was able to obtain a different one for the second brood. In contrast, females who bred twice frequently switched territories (Table 2.1). Only 11 of the 30 females who bred in both the first and second brood made both breeding attempts at the same territory.

Table 2.1. Number of male and female house wrens remaining at the same territory for the first and second brood, switching territories between broods, or failing to breed in the second brood in relation to the number of boxes on the male's territory for the first brood. Treatment did not affect male success at breeding twice during one breeding season (χ^2=0.105, df=3, p=0.991).

Males	Retained territory	Obtained a new territory	No known second brood	Unable to ID male
1 box	8	1	10	3
3 box	10	0	9	2
Females	**Stayed at original territory**	**Moved to a new territory**	**No known second brood**	**Unable to ID female**
1 box	5	10	7	0
3 box	6	9	6	0

Discussion

This study found that male house wrens who were randomly assigned three nest boxes after they obtained a mate invested less in parental care during the early provisioning period than males with only one nest box. Because of their extra boxes, these males had the potential opportunity to attract extra mates, so it is possible that males with three boxes were spending less time provisioning in order to spend more time trying to attract additional mates. We did not measure male singing or other types of courting behavior in this study, so additional work will be needed to determine if such a tradeoff exists. However, data from other studies suggests that males with extra nest boxes do sometimes spend extra time singing. Smith (2005) found that male starlings that were given extra nest boxes sang more and provided less parental care than those with one nest box. In addition, an earlier study on this population of house wrens found that males with multiple nest boxes had higher singing rates during the nestling period than males with one nest box (Dubois 2004).

One apparent problem with this hypothesis is that we found no evidence of increased reproductive success for the three-box males who spent less time provisioning early on. Males with three nest boxes were equally likely to retain their territories and breed twice in one summer compared to males with one nest box, and only one male with three boxes successfully acquired a second female nesting in one of his extra boxes. However, lack of success does not mean that the three-box males were not trying to attract additional females; in fact we observed male wrens singing frequently throughout the entire breeding season (L.A.W., personal observation). We had spaced all three nest boxes in a particular territory relatively close together, and like Dubois (2004) we believe that this close proximity enabled primary females to chase away any competitors their mates might have attracted. In pied flycatchers, female aggression thwarts polygyny when nest boxes are close together (Slagsvold et al. 1999), but this kind of female-enforced monogamy still needs to be tested empirically in house wrens. In addition, it is possible that males with three boxes spent their extra time trying to obtain extra pair copulations rather than a second polygynous mate. Extra pair copulations are relatively common in house wrens (Soukup and Thompson 1997; Soukup and Thomspon 1998), and three box males may also have spent more time guarding their mates to protect their own paternity (Brylawski and Whittingham 2004). An important next step would be to actually measure amount of extra pair paternity achieved by males in each treatment to see if differences in male provisioning rate at the early observation resulted in differences in paternity success.

Male provisioning behavior also changed over time within each treatment. Males with three nest boxes increased their provisioning rates between the early and late observation, while males in the one-box treatment decreased their provisioning rates between two observations. As a result, at the later provisioning observation the pattern of male provisioning was reversed from the early observation. If provisioning at high rates is physically taxing for the birds, one possible explanation for this switch is that one-box males exhausted themselves to provision at higher rates earlier on, leaving them in too poor a condition to maintain that pace throughout the nestling period. In contrast, three-box males who provisioned at lower rates early on might have kept the good condition necessary to maintain, and even slightly increase, their provisioning rate through the late observation. Other alternative hypotheses for the difference between treatments over time are that one-box males began searching for extra mating attempts later than three-box males, or that three-box males, having failed to attract an additional mate despite their early efforts, later began to focus more of their energies on their current nestlings. None of these ideas have been directly tested, and additional research is needed to help address whether these or other hypotheses could explain the change in provisioning behavior over time within treatments.

Although male provisioning behavior differed between treatments in this study, we found no evidence for female differential allocation. Females mated to the supposedly more attractive three box males did not lay more eggs or increase their provisioning rates. These results differ from those of Dubois et al. (2006), who found that females mated to males with three boxes laid significantly larger clutches. Elsewhere (see chapter 4), we show that differential allocation by females in this population has declined systematically over the past 8 years with increasing wren abundance.

Across treatments, male and female provisioning rates were independent of each other at the early feeding observation. However, at the late feeding observation, partner provisioning rates were negatively correlated, suggesting that one partner was compensating when the other partner had a lower provisioning rate. These patterns were consistent within both treatments, suggesting that treatment did not play a role in the way males and females responded to each other. We might expect this type of change in response over time within a population if house wrens provision according to a "negotiation continuum" model of parental care (McNamara et al 1999; Johnstone and Hinde 2006). This model predicts that the degree of an individual's response to changes in provisioning by its partner will depend both on the individual's physical ability to respond and on the quality of the individual's information about brood need or value (Johnstone and Hinde 2006; Hinde and Kilner 2007). It is likely that one or both of these conditions could change between the early and late feeding observation. In this study, male provisioning rates did not increase as the nestlings grew like the female provisioning rates did. If the females responded more strongly than the males to the increased demands of the growing nestlings, this large increase in provisioning by the females might have made additional male help unnecessary at the later observation. Little is known about the exact dynamics of provisioning in the house wren, and more research is needed to understand the specifics of provisioning negotiation in this species.

In this study, provisioning rates were not good predictors of nestling mass ten days after hatching. Male provisioning rates at the early observation were not related to nestling mass or fledging success, despite the fact that other studies on house wrens have found that male care is important at this stage (Bart and Tornes 1989; Johnson et al. 1992). However, these studies permanently "widowed" females by removing their mates, whereas in our study, even the males who provisioned at low rates during our observations still were providing some help to their females. It is possible that we did not have enough variation in provisioning rates to detect a difference in nestling mass or fledging success. It is also possible that nestling mass is not a good indicator of nestling condition at fledging or of future survival in this species, as some studies have found that nestling mass is not always associated with higher nestling condition or survival rates (Magrath 1991; Thompson and Flux 1991; Thompson et al. 1993). Male provisioning rates at the early observation may have had an effect on nestling fitness without having a measurable effect on nestling mass at day 10. This idea is supported by the fact that, contrary to our expectations, overall provisioning rates by both parents were not associated with nestling mass, despite that fact that some nestlings received very little food during our observations compared to others.

Finally, it is possible that our use of provisioning rate is not a good measure of parental investment. Although it is common practice to use provisioning rate as an indicator of parental care, other factors, such as the size of the prey items delivered, might be better measures of parental investment (Schwagmeyer and Mock 2008). These considerations may also vary from year to year as things like food supply and population density change. In addition, birds might behave differently in the presence of observers; or, if provisioning behavior varies greatly throughout a day, our 30 minute observations might not have been long enough to accurately capture individual behavior. Clearly, the assumptions that provisioning rates affect nestling mass and that nestling mass affect nestling survival need to be experimentally tested in this species before we can draw definite conclusions about the effects of provisioning rates on nestling fitness.

Our finding that male house wren provisioning decreases when males have additional mate attraction opportunities is consistent with the idea that males face a tradeoff between parental care and attracting additional mating opportunities in this system, but this study is only a first step. More empirical evidence is needed to determine how tradeoffs between parental care and mate attraction influence lifetime fitness. Understanding this tradeoff is crucial to understanding how sexual conflict mediates asymmetries over investment in parental care between males and females.

CHAPTER 3
BRIGHTER EGGS ARE BETTER IN THE HOUSE WREN

with Thomas Getty

Abstract

Recent work suggests that avian egg color could be a sexually selected signal to males of female condition, genetic quality, or costly female investments in the egg (maternal effects). If female quality or investment is high, this would mean that a male's own investment in the offspring would yield high fitness returns. The primary pigment in blue eggshells is a potentially costly antioxidant, and some studies have found that males increase their parental care when they have more intensely blue eggs in their nest. However, no one has addressed whether egg color influences provisioning in species of birds that lay brown eggs, where the primary pigment in eggshells is a pro-oxidant associated with poor female condition. We performed a cross fostering experiment in the house wren (*Troglodytes aedon*), a species that lays brown eggs, to test whether egg color was related to maternal investment in the eggs and whether male provisioning of chicks was related to egg color. We found that brighter eggs (i.e. those with less brown pigment) were heavier, and that nestlings that hatched from brighter eggs were fed at higher rates by their foster mothers but not their foster fathers. These results suggest that females who lay brighter colored eggs also invest more in those offspring through maternal effects on egg content and post-hatching provisioning, although males do not seem to respond to this potential cue. This study highlights the need for more research into the relationship between brown egg color and parental investment.

Introduction

Bird eggs often vary in color within and between clutches as well as between species. Several hypotheses have been proposed to explain the evolution of colored eggs from the ancestral white form (Kilner 2006). Wallace (1889) originally suggested that colored eggs were an adaptation to conceal eggs from predators. Other explanations (reviewed in Underwood and Sealy 2002) for the evolution of colored eggs include the idea that egg color is under selection by brood parasites or that the colored pigments serve a structural or thermoregulatory function in the eggshell.

A recent hypothesis suggests that variation in eggshell pigmentation within a species could function as a sexually selected signal from female birds to their mates (Moreno and Osorno 2003). The pigments that determine egg color are potentially costly and may reveal female fitness by signaling their antioxidant capacity (McGraw 2005; Soler et al. 2005). For example, biliverdin, the primary pigment in blue eggs shells (Kennedy and Vevers 1976), is an antioxidant (Stocker et al. 1987). Darker blue eggs may signal that a female is of higher quality because she has a high enough antioxidant capacity to allocate extra biliverdin to her eggshells efficiently (Moreno and Osorno 2003; Getty 2006). Alternatively, egg color may signal female investment in those particular eggs if maternal effects (i.e. deposition of yolk nutrients, hormones, antibodies, etc.) are correlated with egg color. If male birds can obtain information about the quality of their mate or her maternal effects on their offspring from observing egg color intensity, differential allocation theory predicts that males should bias their parental investment based on this cue (Burley 1986, Sheldon 2000). This argument has two requirements that need to be tested empirically: female and/or nestling quality must be related to egg color, and males must respond to this signal and bias their parental investment accordingly.

Recent evidence lends support to the idea that egg color is related to female or nestling quality in bird species that lay blue eggs. Empirical work in several different species supports this idea: females in better condition lay darker blue eggs in the pied flycatcher (Moreno et al. 2005, Moreno et al. 2006a,b), eastern bluebird (Siefferman et al. 2006), collared flycatcher (Krist and Grim 2006), and spotless starling (Soler et al 2008). In addition, Morales et al. (2006) found that blue egg color is correlated with aspects of nestling quality in pied flycatchers such as the amount of maternal antibodies in the egg and nestling fledging success. However, the outcomes of experimental studies testing for male response to blue egg color are mixed. Two experimental studies have found that male birds given darker blue eggs contributed more toward nestling provisioning than those given paler eggs in the pied flycatcher (Moreno et al. 2006b) and spotless starling (Soler et al. 2008). In contrast, Krist and Grimm (2006) did not find any relationship between blue egg color and parental investment in the collared flycatcher.

Although several studies have investigated whether male birds in species with blue eggs bias their parental investment based on egg color, no one has tested this idea in a species that lays brown eggs. Protoporphyrins, the primary pigments in brown eggshells (Kennedy and Vevers 1976), are intermediates in the biosynthesis of blood heme (Baird et al. 1975; Burley and Vadhera 1989; but see With 1973). In contrast to the antioxidant blue pigment biliverdin, brown protoporphyrins are pro-oxidants that cause oxidative stress (Afonso et al. 1999, Shan et al. 2000) and are associated with thinner eggshells, female calcium deficiency (Gosler et al. 2005), and pesticide contamination

(Jagannath et al. 2008). Therefore, females that deposit more protoporphyrins in their eggs may be of poorer quality or in poorer physical condition, as Martinez-de la Puente et al. (2007) found in blue tits. Alternatively, if brighter eggs with less brown pigment are larger in size, as Odabasi et al. (2007) observed in chickens, brighter eggshells might signal higher amounts of female investment in those eggs rather than higher female quality or condition per se.

We observed that brown egg color in the house wren (*Troglodytes aedon*) varied both within and between clutches, and we suspected that the logic behind the idea of egg color as a sexually selected signal could also be applied to this species. However, the direction of the effects should be reversed in species that lay brown eggs because of the negative associations found in previous studies between the brown pigment protoporphyrin and both female condition and eggshell quality. We performed a cross-fostering experiment to test the hypothesis that brown egg color is related to female condition or investment and that paternal investment by male house wrens depends on brown egg color. We swapped entire clutches of eggs between nests after clutch completion and recorded egg color and provisioning behavior. Both parents provision nestlings in this species, and male provisioning is especially important for offspring survival early after hatching when females are still brooding the nestlings (Bart and Tornes 1989; Johnson et al. 1992).

If the amount of brown pigment in eggshells reflects some aspect of maternal quality or investment, we predicted that female condition and/or egg mass would be negatively correlated with the amount of brown pigment in the eggshells. We also predicted that males' provisioning rates would be correlated with the color of their foster eggs. If males differentially allocate their parental investment when the return on investment is higher, we would expect males to provision at higher rates when they had eggs with less brown pigment (brighter eggs) in their nests. Alternatively, if eggs with more brown pigment (darker eggs) signal that female investment in that reproductive attempt is likely to be low, males might actually have higher provisioning rates when eggs are darker to compensate for lower female investment. This strategy would be more likely when a male has a low probably of future reproductive success, such as when the male is old or in poor condition.

Methods

Study site and experimental design

We conducted our study in the summer of 2007 at Lux Arbor Reserve in southwest Michigan using a wild population of house wrens. Nest boxes have been provided for wrens at this location since 1998. We inspected boxes at least every other day for nesting activity during the breeding season and checked them every day once laying began.

In order to reduce any possible correlations between egg quality and female quality or maternal effects at laying, we randomly swapped 50 entire clutches of eggs (25 swaps) from wren pairs that were making their first breeding attempt of the season. We swapped eggs between nests that had completed laying on the same day (20 swaps) or within one day of each other (5 swaps). We performed this swap the day after clutch completion (i.e. the first day that we found no new egg in the nest). We also paired swapped clutches so that the clutch size of both nests was the same (16 swaps) or differed only by one egg (8 swaps). One swap was made between nests whose clutch size differed by 3 because they were the only clutches that were completed at that time. We chose to swap entire clutches after clutch completion, rather than one egg at a time as they were laid, because it allowed us to pair two clutches that were approximately the same size (following Moreno et al. 2006b).

When we swapped the eggs, we also measured egg mass and color. The same observer (L.A.W.) scored by eye the relative amount of pigment visible in the shell of each egg on a scale from 1 (bright, nearly white with very little pigment) to 4 (dark, nearly all brown covered with much pigment) at intervals of 0.5. At the same time, we also measured egg reflectance using an Ocean Optics USB 2000 spectrometer running OOIBase32 software. Wren eggs are speckled with brown pigment, and have less pigment at the top of the egg than the bottom, which has so many speckles it is usually close to solid brown. Therefore, we positioned the egg upside down and took the reading at a constant angle (90 degrees) and distance (2 cm) from the bottom of each egg. This was far enough away that even in the cases where the color of the egg was not quite solid, the area viewed by the probe provided an integrated measure of the overall reflectance of the bottom of the egg. The spectrometer automatically averaged three separate readings to make the final spectrum whose reflectance values represented a relative reflectance compared to a white standard (Labsphere Certified Reflectance Standard). We used reflectance data from wavelengths of 400 nm to 700 nm (visible light). We were unable to obtain reliable UV readings, likely due to constraints imposed by our outdoor setup, so we did not include wavelengths from 300 nm to 400 nm in our analyses.

We measured nestling mass ten days after hatching. We captured and measured adults before their eggs hatched and gave them unique combinations of color bands so we could recognize individuals without recapturing them. We used a standard body condition index, the residuals from a regression of mass on tarsus length, as an estimate of adult body condition (Jakob et al. 1996; Schulte-Hostedde et al. 2005). We measured parental provisioning for each nest one time during day 4, 5, or 6 after hatching between 8AM and 10:30 AM. At these times we observed nest boxes from a distance using binoculars for 30 minutes and recorded all visits made by both parents. We used the total number of visits per 30 minutes for each parent divided by the number of nestlings they were feeding as a standard measure of provisioning effort in our analyses. Of our 50 swapped nests, 4 nests were destroyed by predation before hatching, so we do not have provisioning data for these nests. In addition, although we were still able to observe their provisioning behavior, we failed to capture 3 of the remaining 46 females, so analyses that include female condition did not include data from these nests. We also did not include provisioning data from two nests where we were unable to conclusively distinguish between the male and the female during the provisioning observation because we could not find an unobstructed view of the nest box entrance.

Egg color measurement

The egg reflectance spectra exhibited peaks at approximately 630 nm and 700 nm (Figure 3.1), consistent with the presence of the pigment protoporphyrin IX (Courrol et al. 2007). We analyzed the spectra using a principal component analysis (PCA). PCA allowed us to objectively describe variation in the spectra of the eggshells by reducing the large number of correlated reflectance values into a few orthogonal variables (Cuthill et

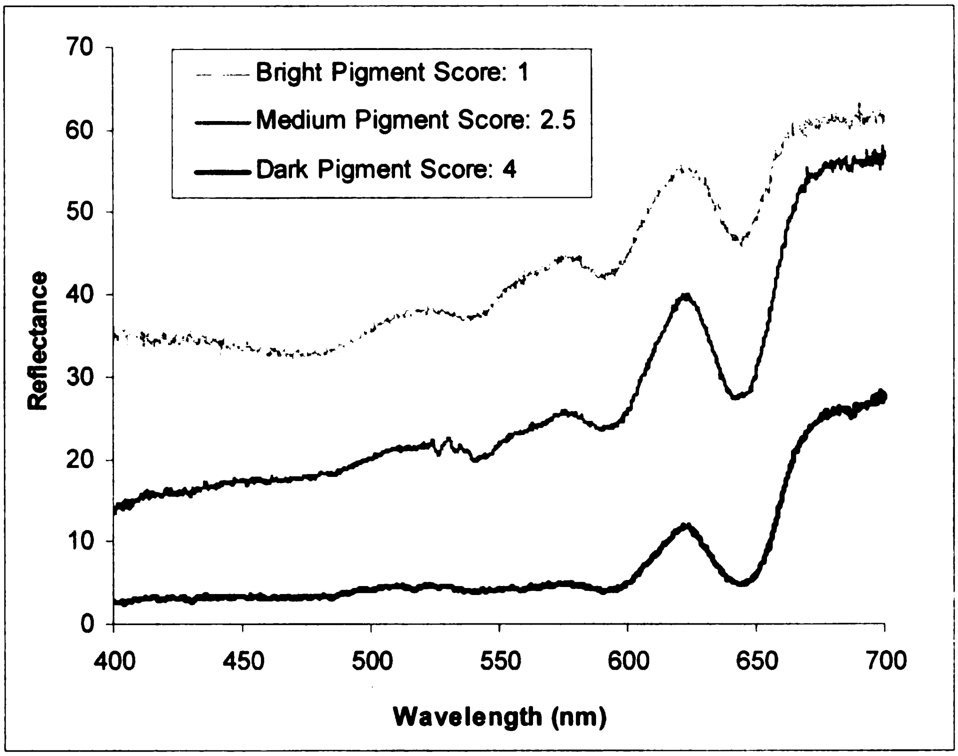

Figure 3.1. Sample reflectance spectra from three eggs representative of three different visible pigment score categories. Eggs with higher reflectance values tended to receive lower pigment scores.

al. 1999). Our original spectral data consisted of reflectance values for wavelengths at 0.38 nm intervals. In order to reduce the number of variables entered into the PCA to a computationally tractable number, we transformed the data to 1.87 nm intervals by calculating the mean reflectance value for every five reflectance measurements (following Aviles et al. 2007). We performed the PCA on the reflectance values at these 1.87 nm intervals for every egg measured during the study (n=345).

When PCAs are performed on spectra from natural objects like eggs, the first principal component (PC1) typically explains around 90% of the variation and has similar loading coefficients across the spectrum (Cuthill et a. 1999). When this occurs, PC1 describes variation in mean achromatic brightness (Endler 1990; Cherry and Bennett 2001). Additional principal components (PC2, PC3, etc.) represent additional variation in spectral shape after variation in brightness is taken into account; as a result they describe variation in chromatic properties like hue and saturation (Endler 1990). Although characteristics of eggshell pigment should influence these chromatic aspects of eggshell reflectance, it is also reasonable to assume that the amount of pigment present in the eggshell would strongly influence PC1 (brightness). Eggshells with no pigment would be white with very high reflectance values, while those with large amounts of pigment would be much browner with lower reflectance values (Figure 3.1). Our visual pigment scores support this argument: the mean value of PC1 for the eggs in each of the seven pigment categories was strongly negatively correlated with the pigment scores we assigned by eye ($F_{1,5}$=34.8, p=0.002, R^2=0.874). This means that eggs with higher PC1 values (brighter eggs) were scored as having less pigment. Because our spectrometric methods reliably captured variation in different aspects of eggshell appearance that were also consistent with our visual scores of the amount of brown pigment, we only used the more objective spectral data in our analyses. We will refer to the values of the first three principal components, which together explained over 99% of the variation between spectra in this study, as together representing variation in egg color.

Statistical Analysis

We used the data from our PCA of the reflectance spectra to examine the relationship between egg color and maternal investment. We used general linear mixed effect models with value of PC1, PC2, or PC3 for each egg as our response variables. Our two predictor variables were female body condition and egg mass, with nest as a random effect to control for non-independence of eggs within a nest.

We assessed whether the provisioning rates of female or male foster parents were related to mean egg color at the level of the clutch, rather than at the level of the individual egg as in the previous analysis. In these models, our response variable was the provisioning rate per nestling by either the male or the female, measured as the total number of visits in 30 minutes divided by the number of nestlings in the nest. Our predictor variables were the mean values of PC1, PC2, and PC3 for the foster eggs in each nest.

Our random cross-fostering manipulation did not completely decouple the association between original and foster egg color, as the mean values of PC1 and PC2 for foster eggs were significantly correlated with those of the original eggs in a nest (PC1: $F_{1,41}=12.1$, $p=0.001$, $R^2= 0.228$ PC2: $F_{1,41}=43.8$, $p<0.001$, $R^2= 0.516$). We suspect that this correlation, which Krist and Grim (2007) also encountered in a similar experiment using blue eggs, occurred because by necessity we swapped eggs from nests that were completed on the same day and with similar clutch sizes. Therefore, we could not be sure whether relationships between provisioning rates and egg color are due to egg color alone or to some aspect of female or male quality that is correlated with egg color.

We addressed this issue in two ways. First, we ran the models again with original egg color (PC1 and PC2) and female or male condition included as additional predictors along with manipulated egg color (PC1 and PC2). If differences in provisioning rates only occurred because parents that had brighter eggs received brighter foster eggs, we would expect there to be no effect of manipulated egg color in these models after the effect of original egg color is taken into account. If, however, differences in provisioning rates are due to some property of the eggs themselves and not parental condition or quality, we would still expect to see an effect of foster egg color on provisioning rates in these models. As a second strategy to address this issue, we also examined the relationship between provisioning rates and the difference between foster and original egg color. If parental provisioning is related to egg brightness, we would expect parents who received foster eggs that were brighter than their original eggs to provision at higher rates compared to those who received foster eggs that were darker than their original eggs, regardless of the actual color of their original eggs. We used general linear models with either male or female provisioning rate as response variables and with the difference between the mean values of PC1, PC2, and PC3 for foster eggs minus original eggs as predictor variables. We performed all statistical analyses using the program R (R Development Core Team 2006).

Results

PC1, PC2, and PC3 together explained 99.2% of the variation in egg reflectance across clutches. PC1 explained most of the variation between spectra (88.1%) and was constant with positive loading coefficients at all wavelengths (Figure 3.2). Therefore, higher PC1 values correspond to higher overall reflectance (i.e. brighter eggs with less brown pigment). PC2 (8.45%) and PC3 (2.65%) explained most of the remaining variation between the spectra. PC2 and PC3 had loading coefficients that varied according to wavelength, so they represent additional chromatic variation between the spectra.

Eggs that were brighter (higher values of PC1) were also heavier ($F_{1,239}$=7.62, p=0.006). PC2 and PC3 had no relationship with egg mass (PC2: $F_{1,239}$=2.20, p=0.139; PC3: $F_{1,239}$=1.30, p=0.255). In addition, female condition was not related to any of the clutch mean PC values (PC1: $F_{1,40}$=0.260, p=0.613; PC2: $F_{1,40}$=0.163, p=0.689; PC3: $F_{1,40}$=0.234, p=0.631). The mean mass for a clutch of eggs did not predict the mean mass of the nestlings from those eggs 10 days after hatching ($F_{1,43}$=0.404, p=0.529).

Male provisioning was not related to foster egg color for any of the PCs (PC1: $F_{1,39}$=0.574, p=0.454; PC2: $F_{1,39}$=0.004, p=0.948; PC3: $F_{1,39}$=0.045, p=0.832). Female provisioning rate increased significantly as the mean PC1 value (brightness) of her foster eggs increased ($F_{1,39}$=4.14, p=0.049; Figure 3.3). Two data points seem to have a strong effect on this correlation (Figure 3.3), but we had no reason to exclude them from our analysis. Female provisioning rate had no significant relationship with the mean value of PC2 ($F_{1,39}$=2.77, p=0.104) or PC3 ($F_{1,39}$=2.97, p=0.093) for her foster eggs. Male and

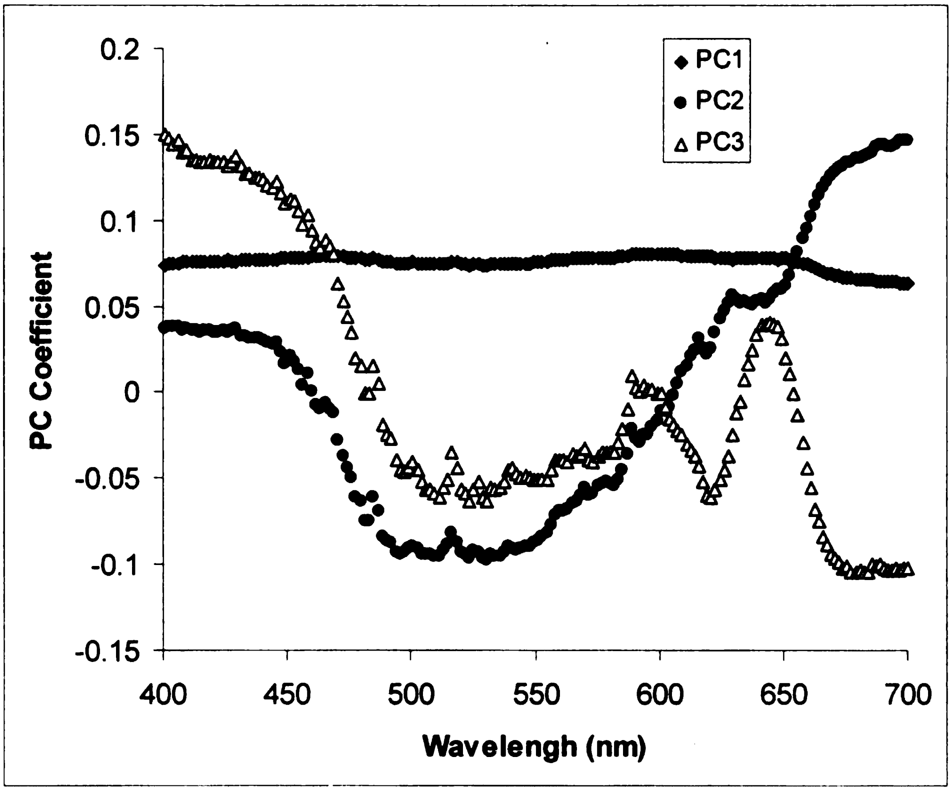

Figure 3.2. Principal component (loading) coefficients as a function of wavelength for the three primary principal components from the principal component analysis performed on the reflectance spectra of 345 eggs. PC1 (black diamonds) indicates principal component 1, which represents variation between eggs in achromatic brightness and accounts for 88.1% of the variation between spectra. PC2 (principal component 2; grey circles) and PC3 (principal component 3; open triangles) represent additional variation in chromatic aspects of egg appearance such as hue and saturation.

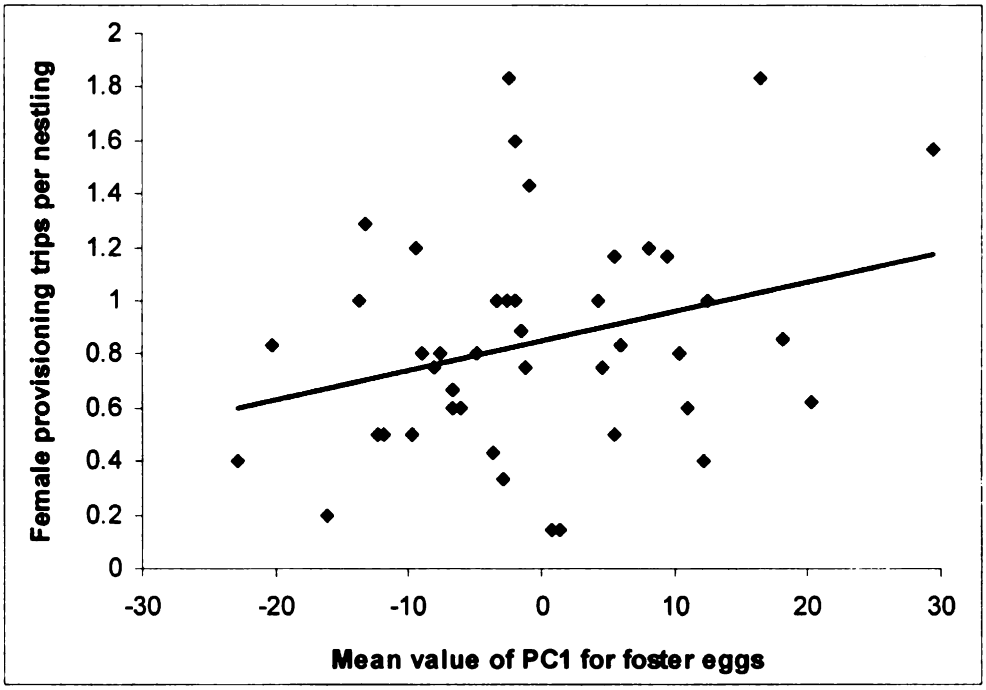

Figure 3.3. Relationship between mean PC1 values (egg brightness) of foster eggs and female provisioning trips per nestling during a 30 minutes observation period. Females who had brighter eggs in their nests provisioned at higher rates ($F_{1,39}$=4.14, p=0.049).

female provisioning rates were not correlated with each other ($F_{1,41}$=0.093, p=0.762, R^2=0.002).

In our provisioning models that included PC values for both original and foster eggs as well as parent condition, the results for PC1 were similar to the models that only included foster egg color. Females provisioning rate was significantly positively related to the mean PC1 value of her foster eggs ($F_{1,35}$=7.58, p=0.009) but had no relationship with the mean PC1 values of her original eggs ($F_{1,35}$=0.062, p=0.804) or female body condition ($F_{1,35}$=0.389 p=0.537).

Although female provisioning rate was not related to the mean PC2 value of her foster eggs ($F_{1,35}$=0.038, p=0.846), it was positively related to the PC2 value of her original eggs ($F_{1,35}$=4.407, p=0.043). Females who originally laid eggs with high PC2 values provisioned their foster nestlings at higher than average rates (Figure 3.4). These additional models also confirmed that male provisioning rates were not related to any of the predictor variables we tested (PC1 original: $F_{1,31}$=0.047, p=0.830; PC1 foster: $F_{1,31}$=1.404, p=0.245; PC2 original: $F_{1,31}$=0.125, p=726, PC2 foster: $F_{1,31}$=0.343, p=0.562; male condition: $F_{1,31}$=0.599, p=0.445).

In our models comparing the difference in color between original and foster eggs, the change in PC1 was positively correlated with female provisioning rate ($F_{1,39}$=5.83, p=0.021; Figure 3.5). Females whose foster eggs were brighter than their original eggs provisioned at higher than average rates, while those whose foster eggs were less bright than their original eggs provisioned at lower than average rates. The difference between foster and original egg color for PC2 and PC3 was not related to female provisioning rate

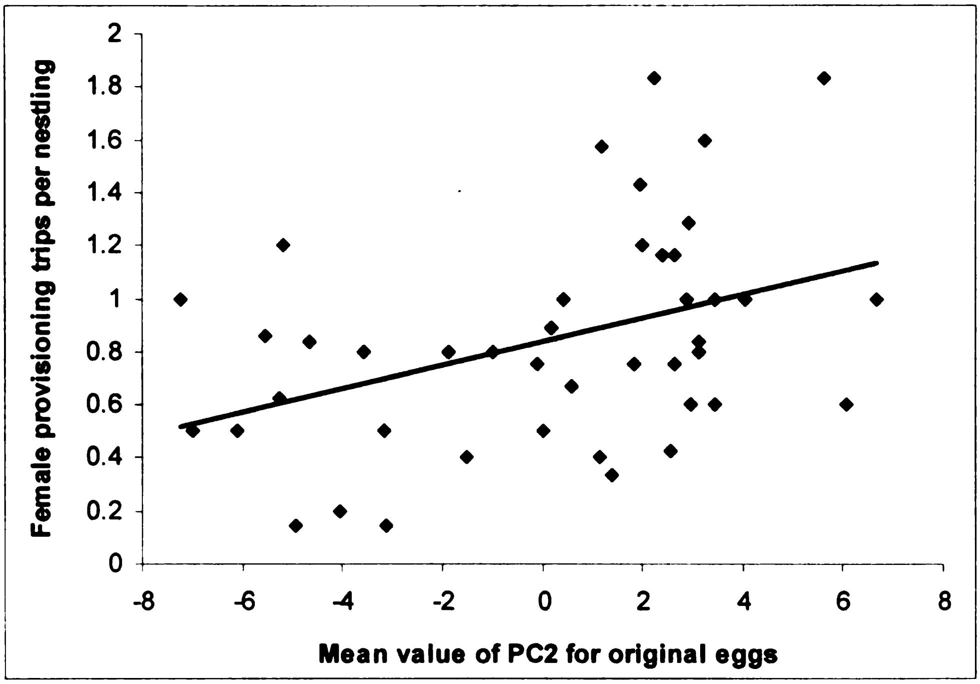

Figure 3.4. Relationship between mean PC2 values of original eggs and female provisioning trips per nestling of her foster nestlings during a 30 minutes observation period. Females who had laid eggs with higher values of PC2 provisioned their foster nestlings at higher rates ($F_{1,35}$=4.41, p=0.043).

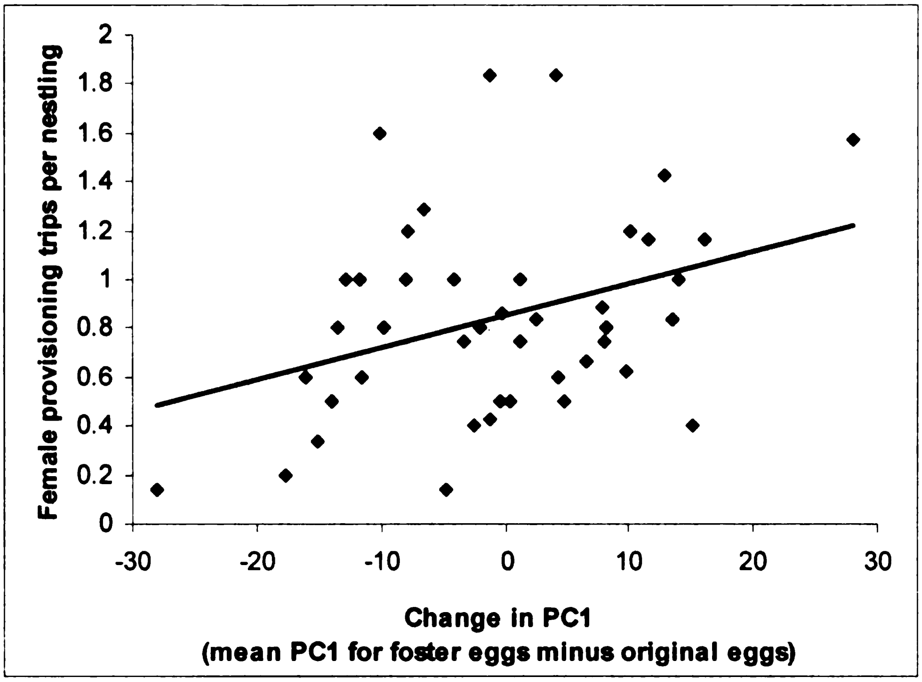

Figure 3.5. Relationship between the change in PC1 values (mean PC1 values for foster eggs minus original eggs) and female provisioning trips per nestling for foster nestlings during a 30 minute observation period. Females whose foster eggs were less bright than their original eggs provisioned at lower rates those whose foster eggs were brighter than their original eggs ($F_{1,39}$=5.83, p=0.021).

(PC2: $F_{1,39}$=0.933, p=0.340; PC3: $F_{1,39}$=0.007, p=0.935) and no aspects of the difference in egg color were related to male provisioning rate (PC1: $F_{1,39}$=0.849, p=0.363; PC2: $F_{1,39}$=0.478, p=0.494; PC3: $F_{1,39}$=0.906, p=0.765).

Discussion

This study provides evidence that the amount of brown pigment in eggshells is related to maternal effects on the offspring inside those eggs. We found that brighter house wren eggs (i.e. those with less brown pigment) were heavier, and that nestlings from clutches of eggs that were brighter on average and in comparison to the original clutch were provisioned at higher rates by their foster mothers. Even though our cross-fostering manipulation did not completely eliminate the potential correlation between egg color and female quality, the positive relationship between the change in egg color and female provisioning rates suggests that at least part of the correlation between egg color and female provisioning is due to maternal effects on nestling quality, rather than female quality itself. In addition, female provisioning rates were not related to our index of body condition or to the brightness (PC1) of the eggs they originally laid, providing further evidence that the relationship between foster egg brightness and female provisioning rates is due to some aspect of the foster eggs or nestlings, rather than characteristics of the female. Although we did not expect female provisioning rates to be affected by the color of their foster eggs, we speculate that nestlings from brighter eggs were of higher quality, and therefore better at begging, than those from darker brown eggs.

Nestlings from heavier eggs, which in this study were also brighter in color, may elicit more provisioning from their parents because higher egg mass is usually associated with increased growth and survival in birds (Willaims 1994). In this study, mean nestling mass at day 10 was not correlated with the mean mass of the eggs they hatched from. However, we did not measure nestling mass at day 4-6, which is when we made our provisioning observation, so we do not know the status of nestlings at that time. In a study on house wrens, Styrsky et al. (1999) found that egg mass in the first brood was correlated with nestling mass early after hatching, but by day 10 the effect had disappeared. It is also possible that nestlings from larger eggs beg more effectively even if they do not have higher mass. In addition, begging ability could be related to the level of antibodies (Morales et al. 2006), antioxidants (Helfenstein et al. 2008), or hormones (Schwabl 1996) that females allocated to eggs. Females who lay brighter eggs could also have allocated larger amounts of these beneficial compounds in addition to the higher overall mass of brighter eggs that we have demonstrated in this study. More research is needed to determine exactly what aspects of nestling and female quality are related to brown egg color. Studies directly measuring the relationship between egg color and begging intensity, for example, would help our interpretation of why female provisioning is higher for nestlings from brighter eggs.

Interestingly, females who originally laid eggs with positive values of PC2 provisioned their foster nestlings at higher rates, regardless of the color of the eggs the foster nestlings came from. PC2 had high positive loadings at wavelengths above 600, so higher values of PC2 could be interpreted as eggs that appear redder. Since high values of PC2 in eggs seem to predict higher provisioning rates in the females that laid them, this component of egg color could be related to some aspect of female quality. This relationship merits further research to determine what characteristics of eggshells influences redness and what specific female characteristics are related to redder eggs.

No aspect of egg color predicted male provisioning rates in this study, which is surprising given the positive relationships between egg color and female provisioning rates. However, male and female birds do not necessarily use the same provisioning strategies, and other studies have found differences between males and females in their response to nestlings (MacGregor and Cockburn 2002; Mock et al. 2005; Ardia 2007). Even if nestlings from brighter eggs are more effective beggars, males and females could respond to their begging in different ways depending on the fitness value of a such as response (Johnstone and Hinde 2006).

It is also possible that some aspect of male investment that we did not measure is dependent on egg color or correlated traits. For example, males with brighter colored foster eggs might continue to feed their nestlings later into the season rather than abandoning them to search for a mate for the second brood. Male house wrens provide relatively more parental care for nestlings during the second breeding attempt of the season than the first (Dubois 2004), so perhaps males would be more responsive to nestling begging if a similar experiment were performed during their second breeding attempt

We do not believe the lack of male response to egg color was the result of males not seeing the eggs. Male house wrens frequently enter and inspect the nest when females are away (L.A.W., personal observation). Even though the interior of a nest box is relatively dark, some light enters through the opening. The relatively lower light levels inside nest boxes does not appear to be an obstacle to assessing egg color for male pied flycatchers (Moreno et al. 2006b) or spotless starlings (Soler et al. 2008). In both of these cavity nesting species, males provisioning rates were influenced by experimentally manipulated egg color. The majority of the work examining these types of questions has been performed in cavity nesting species, and it would be interesting to see if these patterns also hold up in open-nesting species, where spectral properties and predation pressures would be different.

The results of this study suggest that nestlings from brighter eggs have the advantage of greater maternal investment, most likely through maternal effects on egg content. These findings represent an intriguing first step into the investigation of brown egg color as a potential cue of female investment in their nestlings. The correlations we observed between egg brightness and nestling quality set the stage for the evolution of egg brightness as a sexually selected cue in brown eggs. However, there is currently no evidence that males respond to this potential cue in species that lay brown eggs. It is possible that egg color does not function as a sexually selected signal in species that lay brown eggs, although more research is needed to determine how brown egg color is related to parental investment for both sexes. If males of species that lay brown eggs truly do not use egg color to assess female quality or investment like they do in species that lay blue eggs, understanding the reasons for this difference will increase our knowledge of how evolution influences sexually selected traits.

CHAPTER 4
DIFFERENTIAL ALLOCATION IN THE HOUSE WREN DECLINES SYSTEMATICALLY ACROSS YEARS AS POPULATION DENSITY INCREASES

with Natalie S. Dubois and Thomas Getty

Abstract

Differential Allocation theory suggests that iteroparous organisms should increase their parental investment when they have an attractive mate who increases the reproductive value of offspring produced from that breeding attempt. Studies testing for differential allocation in wild populations have yielded variable and conflicting results. We present data from a wild population of house wrens (*Troglodytes aedon*) in which female response to male quality changed over time. For six years, we monitored the magnitude of parental investment by female wrens whose mates were randomly assigned to either a high quality (three nest boxes) or low quality (one nest box) territory. Early on, females mated to a male randomly assigned one nest box in his territory had significantly smaller clutches than females mated to a male randomly assigned three nest boxes in his territory. However, the difference in clutch size between females in the two treatments declined over time, as the clutch size of one-box females increased systematically to equal those of three-box females. In order to determine what might be causing this diminishing female response, we looked for a relationship between year and other factors that might change systematically over time, including weather, female experience, and population density. Only population density changed systematically across years. The differential female response was strongly negatively correlated with local wren density. This pattern is consistent with the hypothesis that with increasing

density, the expected value of future breeding opportunities and the reproductive value of future offspring decline. Future studies of differential allocation and sexual selection should consider the effects of population-level characteristics like density.

Introduction

Reproductive effort is costly; by definition it reduces expected future reproduction (Trivers 1972; Clutton-Brock 1991; Stearns 1992). Consequently, iteroparous organisms, with repeated bouts of reproduction, face a fundamental life history tradeoff between investment in current and future reproduction. If the marginal fitness return on reproductive effort varies between breeding attempts, organisms should invest relatively less when the returns are low in order to increase investment in future breeding attempts when the returns might be higher. In sexual organisms, the return on reproductive investment can depend on the quality of the mate in a particular breeding attempt. An attractive high quality mate can improve the return on investment either directly, by providing better parental care and resources, or indirectly, by providing genetic benefits to the offspring (Sheldon 2000). If opportunities for future breeding attempts with higher quality mates are abundant, individuals mated to unattractive low quality mates might be better off saving reproductive effort for better mates later. This idea is called the Differential Allocation Hypothesis (Burley 1986, 1988).

Differential allocation is gaining increasing recognition as an important component of sexual selection (Sheldon 2000; Head et al. 2006). Although there has been some empirical support for differential allocation in a variety of species (e.g. Osorno et al. 2006, Loyau et al. 2007; Roed et al. 2007; Skinner and Watt 2007), other studies testing for differential allocation yield negative or conflicting results (e.g. Cooper and Ritchison 2005; Hargitai et al. 2006; Ferree 2007, Nakagawa et al. 2007). Even studies using similar manipulations on the same species can produce different results. For example, Limbourg et al. (2004) found that female blue tits mated to males whose attractiveness was experimentally reduced had lower provisioning rates than females mated to control males, causing their nestlings to have shorter tarsus lengths. In contrast, Johnsen et al. (2005) found that male attractiveness had no overall impact on female provisioning rate or nestling tarsus length in a different population of blue tits. What are we to make of this inconsistency?

It is possible such differences among studies result from random noise, variation in abiotic conditions, or slightly different experimental methods. However, some of the apparent noise could also be due to overlooked differences in demographic characteristics. Indeed, Johnsen et al. (2005) found that female age influenced differential allocation in their study population. Even though there was no overall effect of male attractiveness on female provisioning, the results changed when older females were excluded from the analysis. Younger females with less attractive males did indeed provision at lower rates than younger females with more attractive males. This pattern of older females not reducing current effort when paired with less attractive males is consistent with the hypothesis that older females have a lower probability than younger females of improving their breeding opportunities the next year.

Other demographic characteristics, such as population density, could also have important effects on differential allocation. The Differential Allocation Hypothesis focuses on the probability of having future breeding attempts with higher quality partners. This probability will be influenced by not only by the probability that a successful future breeding attempt will be with a partner of higher quality than the current one, but also by the probability that there is a successful future breeding attempt at all. All else being equal, the first component should increase if the current partner is below average. However, we might expect the second component to decrease if the female is old, or if the population density is high relative to the number of available nesting sites. For example, a female with a low quality mate in a habitat with 100 nest sites has a lower expectation of successfully breeding with a higher quality male in the future if she is competing with 200 other females than if she is competing with 100 other females. An additional demographic consideration is that in a growing population, the reproductive value of future offspring is discounted by the population growth rate (Fisher 1958). In a growing population, fewer offspring now can be more valuable than more offspring later.

Many studies have found that clutch size is negatively correlated with density (Perrins and McCleery 1989; Both 1998; Wilkin et al. 2006; Mallord et al. 2007), but researchers have not empirically explored how density might interact with mate quality to affect female investment in reproduction. Despite the important influences of density on many types of animal behavior, scientists studying sexual selection have tended to focus exclusively on a behavior of interest, overlooking possible interactions with population density (Kokko and Rankin 2006). One likely reason is that behavioral studies are often conducted on a single population over one or two years. However, interactions between density and a behavioral response will only become apparent using multiple populations or a single population monitored over multiple generations for which density varies.

We studied variation in differential allocation in a single population of house wrens (*Troglodytes aedon*) over six years. Male house wrens defend breeding territories containing one or more preexisting cavities and are facultatively polygynous (Johnson 1998). The number of cavities on a male's territory, a trait that is related to male competitive ability, influences female reproductive decisions (Dubois et al. 2006; Eckerle and Thompson 2006). Therefore, we consider it a component of "male attractiveness" and use that term hereafter to refer to the number of cavities on a male's territory. Previously, Dubois et al. (2006) reported results from this population demonstrating that females mated to males who were randomly assigned territories containing surplus nest boxes produced larger clutches than females mated to males given only one nest box. We continued to study this population to address new questions. To our surprise, the female differential allocation effect declined over the six years of combined studies. In this paper we describe the pattern and provide correlative evidence consistent with the hypothesis that differential allocation is sensitive to local population density.

Methods

We conducted our study from 2001-2002 and 2004-2007 at Lux Arbor Reserve, a 529 ha site in southwest Michigan. The study site contains fragmented habitat including deciduous and coniferous forests, agricultural fields, open fields, and wetlands. This area has few natural cavities because the forests consist of mostly younger, secondary growth trees, and because dead trees are periodically removed. Therefore, prior to 1998, the year that nest boxes were first erected as this site, there were very few wrens nesting in the area. The local population of nesting wrens began to grow after nest boxes were provided. At the same time, the abundance of house wrens across the region was also growing; according to the North American Breeding Bird Survey (Sauer et al. 2008; http://www.mbr-pwrc.usgs.gov/bbs/), house wren abundance in the state of Michigan increased significantly between 1998 and 2007.

The current study began in 2001, by which time we had established 53 territories that each contained three nest boxes. Boxes within a territory were situated within 15 m of each other, and territories were spaced approximately 100 m apart so that male wrens could only control boxes on one territory at a time. We held the number of available territories steady at 53 from 2001 to 2007. Every year we removed the boxes after the breeding season, emptied old nests, and returned the boxes before the wrens returned in the spring, replacing or repairing damaged or worn out boxes

Before the wrens arrived each spring, all territories contained three nest boxes, but only a single nest box was available to the wrens. The other two boxes were plugged using rubber stoppers to prevent access to the nest box. In 2004-2007, after both a male and female had arrived on a territory, we randomly assigned the pair to either the single or multiple nest box treatment. For territories in the multiple box treatment, we removed the rubber stoppers from the two extra nest boxes, providing the breeding pair with access to three boxes. We considered a territory to be occupied by a breeding pair once the cup nest was lined with soft material such as feathers, moss, or grass. Although both males and females build the stick portion of the nest (Alworth and Scheiber 2000), this lining behavior is uniquely performed by the female (Kendeigh1952).

Our experimental design ensured randomization of both male and female quality with regard to the treatment of single versus multiple nest boxes in male territories.

Although differences in quality surely existed among individual birds, the average condition of males and females should have been the same in both treatments, since the treatments were randomly assigned after settlement. As a result, we could compare reproductive investment between treatments independent of actual individual quality. The increase in available boxes within a territory should not have lowered the female's perception of population density because the newly opened boxes were spaced close together within the already established territory of her mate. Therefore, the newly opened nest boxes did not increase the number of nesting sites that were available for additional breeding pairs. Over the course of the study, additional breeding pairs settled in these newly opened boxes less than 1.5% of the time (4 instances out of 267 available boxes).

A slightly different design was used in 2001-2002 (described in Dubois et al. 2006); the primary difference was that the stoppers were removed from the surplus nest boxes after male settlement but before female arrival. We modified the design in 2004-2007 in order to rule out the possibility that females in better condition preferentially settled with males in territories with multiple nest boxes. This change in design between 2002 and 2004 did not result in a qualitative change in female response between the two years (Figure 4.1), supporting the argument that female sorting in 2001-2002 did not have a major influence on the observed differences in clutch size. Boxes were provided for the wrens in 2003, but this manipulation of box number was not performed that year.

We checked nests at least every other day and recorded the status of the nest, including clutch size. We captured, banded, and measured females during incubation. In our analyses, we only included first mated females on the first nesting attempt in each territory (n=242). Consistent with Dubois et al. (2006), polygyny was rare in this population, and never occurred until well after the first mated female had completed laying her clutch. For females that bred in our study population during multiple years (n=35), we only used data from one randomly selected year to ensure statistical independence.

We estimated breeding population density by counting the total number of territories (out of 53) that were occupied by both a male and a female during the first breeding season, before any wrens attempted a second brood. We considered females experienced if they had bred in our population in a previous year and naïve if they were born in our population the previous year or if they were unbanded at capture. Unbanded females had not bred previously at our study site, but it is possible they bred elsewhere in previous years. The few females (less than 10%) whose band status was unknown, usually because their nest was destroyed by predation before they could be identified, were not included in the calculation of female experience or in models that included female experience.

We also examined the effects of weather conditions on variation in differential allocation. We used the average of spring (March through May) monthly mean values of the North Atlantic Oscillation (NAO) index as an integrated measure of general weather trends (Barnston and Livezey 1987). The NAO influences temperature and rainfall patterns in Europe and North America (Thompson and Wallace 2001), and has been shown to affect avian productivity in North America (Nott et al. 2002; Jones et al. 2003; Weatherhead 2005). We obtained NAO index values from the National Oceanic and

Atmospheric Administration's Climate Prediction Center (http://www.cpc.ncep.noaa.gov/data/).

We compared the response of females to male quality across years using an ANOVA with clutch size as the response variable and treatment, year, and the interaction between treatment and year as predictor variables. Because Johnsen et al. (2005) found a significant interaction between treatment and female age, we initially included female experience and the interaction between experience and treatment in this model. However, we removed them for the final model because they were not significant predictors of clutch size and including them decreased the fit of the model.

Because year itself is not a biologically significant variable, we also tested for demographic and abiotic factors that might vary by year. To do this, we performed a second ANOVA with year as the response variable. Our predictor variables were density, measured as the number of occupied territories, weather conditions, measured as the mean spring NAO index value (March-May), and population-level female experience, measured as the proportion of naïve females. We performed all statistical analyses using the program R (R Core Development Team, 2006).

Results

Females mated to a male randomly assigned three nest boxes in his territory laid significantly larger clutches than females mated to a male with one nest box in his territory ($F_{1,203}$=4.55, p=0.034). However, female response to manipulated male attractiveness also varied among years as evidenced by a significant treatment by year interaction in our initial ANOVA ($F_{1,203}$=4.34, p=0.038). In earlier years, the difference in mean clutch size between treatments tended to be larger, while in later years there was no difference between the two treatments (Figure 4.1). To determine how changes in the clutch size of females in each treatment affected this interaction, we performed ANOVAs analyzing the relationship between year and mean clutch size separately for females in each treatment group. The clutch size of females in the one box treatment increased significantly over time ($F_{1,4}$=16.4, p=0.015). The clutch size of females in the three box treatment did not change significantly, but exhibited a marginally non-significant pattern of decreasing with year ($F_{1,4}$=7.57, p=0.051), although the magnitude of the decrease was smaller than the increase in clutch size among one box females.

Breeding bird density in our study population increased significantly over time ($F_{1,2}$=32.8, p=0.029; Figure 4.2). This increase parallels state and regional increases estimated from the USGS Breeding Bird Survey (Sauer et al. 2008). Spring NAO index values ($F_{1,2}$=4.86, p=0.158) and population-level female experience ($F_{1,2}$=6.51, p=0.125) were not related to year. In addition, individual female experience did not influence clutch size ($F_{1,181}$=2.07, p=0.152) and had no interaction with treatment ($F_{1,181}$=1.14, p=0.287) in the initial version of our first model. Because only density was correlated with year, and because year interacted significantly with treatment to influence clutch size, we performed a post hoc linear regression between density and the difference between treatments in mean yearly clutch size (Figure 4.3). Differential allocation and density were strongly negatively correlated (r^2=0.811, $F_{1,4}$=17.2, p=0.014), although the average yearly clutch size for the population as a whole was not related to population density ($F_{1,4}$=1.79, p=0.252).

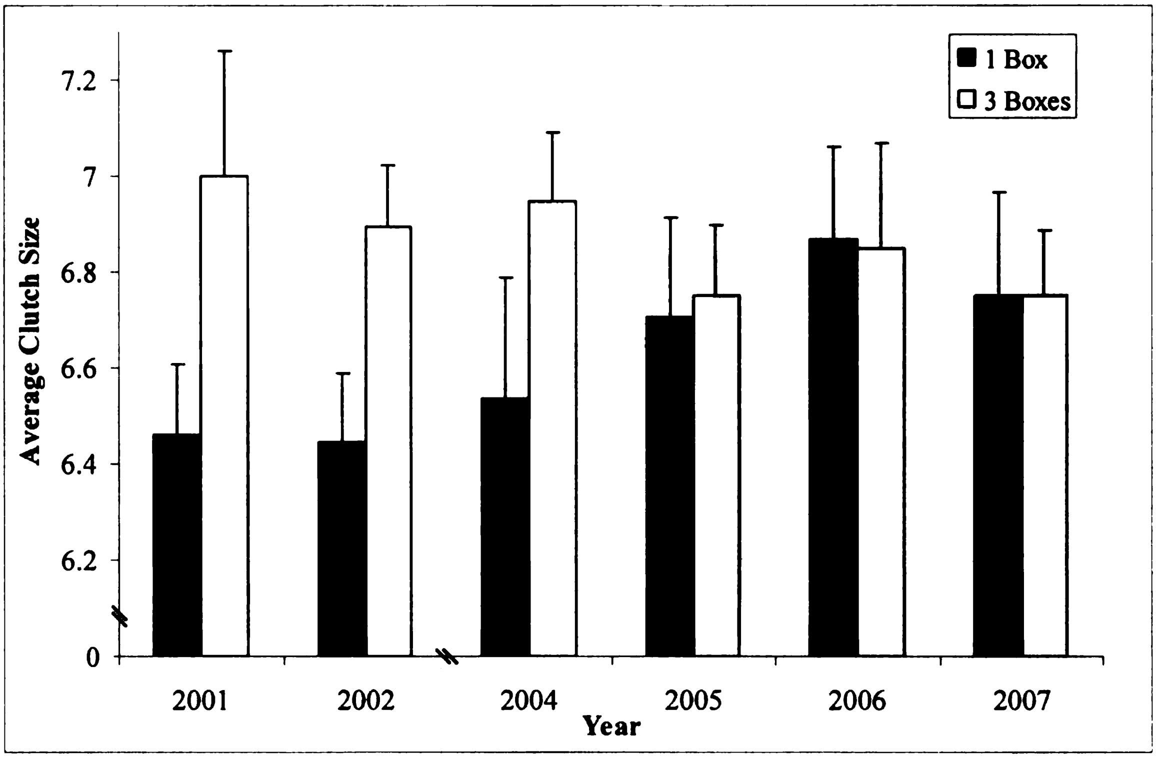

Figure 4.1. Mean clutch size (± s.e.) for females mated to males with one nest box (filled bars) and females mated to males with three nest boxes (open bars) by year (treatment by year interaction: $F_{3,203}$=4.34, p=0.038).

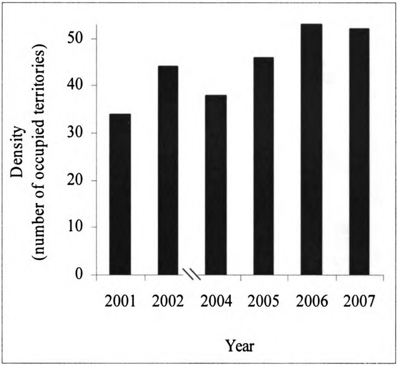

Figure 4.2. Density, measured as the number of occupied territories (out of 53), each year of the study. Density increased significantly over time ($F_{1,2}$=32.8, p=0.029).

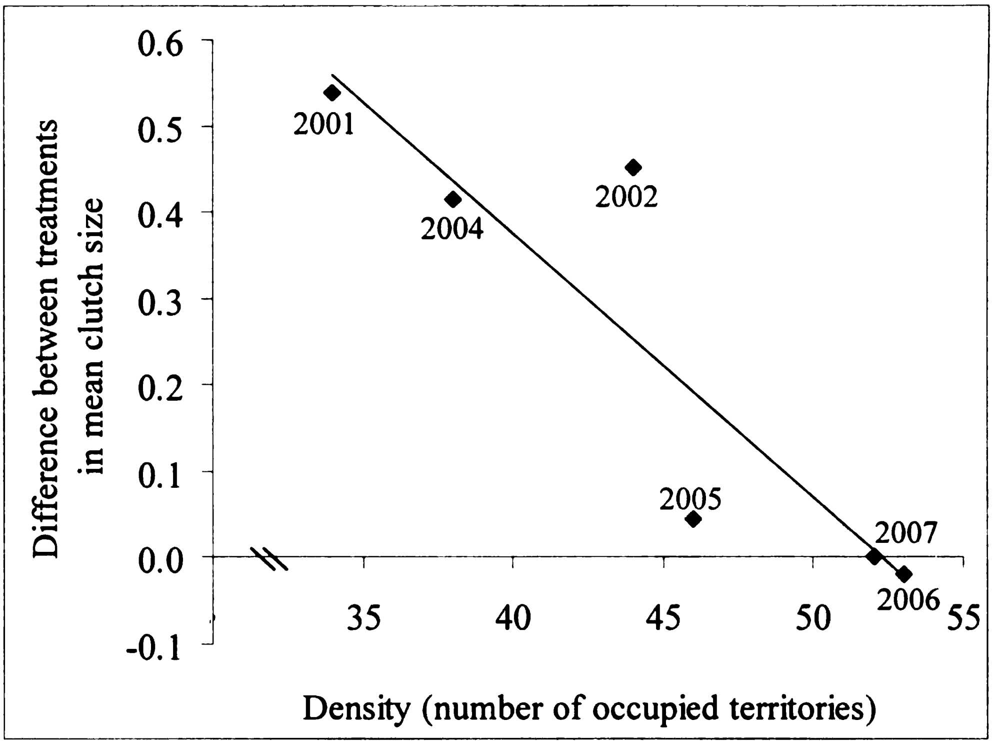

Figure 4.3. Relationship between density, measured as the number of occupied territories (out of 53), and differential allocation, measured as the difference between treatments (three boxes minus one box) in mean clutch size (r^2=0.811, $F_{1,4}$=17.2, p=0.014).

To determine if the correlation between differential allocation and density might be affected by the minor difference in methods between 2001-2002 and 2004-2007, we also analyzed the two groups of years separately. We performed the same linear regression between density and the difference between treatments in mean clutch size using only the years from 2004-2007. In this more limited analysis we found the same pattern as when all the years were included, although the trend was marginally non-significant ($r^2=0.892$, $F_{1,2}=16.4$, $p=0.056$), likely due to the loss of statistical power from decreasing our sample size from 6 years to 4. This pattern of increased differential allocation with lower density also held for 2001-2002, but with only two data points we could not statistically test the pattern for these two years by themselves.

Discussion

Differential allocation to clutch size in response to male attractiveness declined across years as density increased in this population of house wrens (Figure 4.3). Females mated to more attractive (three-box) males consistently laid large clutches. The declining difference was due to a decline in the response of females mated to less attractive (one-box) males; as the local and regional density of house wrens increased over the years, one-box females reduced their clutch sizes less and less relative to three-box females (Figure 4.1).

We have no reason to think that time itself should influence differential allocation; instead, we propose that increasing density over time explains the negative relationship between year and differential allocation. If the decline in differential allocation over time was best explained by the slight change in methodology between

2002 and 2004, we would have expected to see a dramatic change in differential allocation between 2002 and 2004 in Figure 4.1 and two distinct groupings by methodology in Figure 4.3. Contrary to these predictions, we see a relatively continuous decline in differential allocation in Figure 4.1 and a pattern consistent across all 6 years in Figure 4.3, suggesting that the change in methods is not an adequate explanation for the decline in differential allocation over time. In fact, the most dramatic change seemed to occur between 2004 and 2005, two years with identical experimental designs. We observed no obvious environmental changes between 2004 and 2005 that might explain this change, but wren density did increase by a relatively large amount between those two years.

We have not manipulated the density of breeding birds, so the decline in differential allocation with increasing density is correlative. It is possible that some other causal factor covaries with time, density, and differential allocation, explaining the negative correlation found between density and differential allocation in this study. We performed post hoc tests looking for other possible covariates of time that could have caused the change in differential allocation. However, we could find no other covariates of time after testing winter NAO values, local precipitation and temperature during the breeding season, yearly local precipitation and temperature, female size, female return rate, and nestling mass. Nor were we able to find any other covariates of either differential allocation or density.

Unlike Johnsen et al. (2005), we found no interaction between treatment and female age. In addition, density had no effect on overall population clutch size, in contrast to previous studies on other species that have found a negative relationship between population density and clutch size (but see Sinclair 1989). This lack of a direct density effect on clutch size is perhaps not surprising, given that we placed nest box clusters far enough apart that there should be little, if any, competition between neighboring wrens for resources within territories. In this experimental population, increasing wren density should have a much greater impact on competition for nest boxes than on competition for food.

Females in the two treatment groups allocated their clutch sizes differently when the density of wrens was low relative to the density of nest boxes. At relatively low densities, females whose mates had one nest box seemed to be withholding reproductive effort by having smaller clutch sizes compared to years when density was high. Withholding reproductive effort for future reproductive attempts only makes sense if there are reasonable expectations for better future reproductive attempts. One possible explanation for the increase in clutch size of one box females as density increased is that actual or perceived competition for mates and nest boxes is greater in high density years. Recent work suggests that females should increase their clutch size when their future access to mates is uncertain (Heubel et al. 2008). If this is the case, we would predict that females mated to less attractive (one-box) males should invest maximally in reproduction during high density years despite the low attractiveness of their mate because they perceive a low future probability of acquiring a breeding territory or higher quality mate. However, in lower density years when future reproduction is more certain, we would predict that females should decrease their investment in clutch size when they have a comparatively less attractive mate because they perceive a higher probability of better breeding opportunities the next year.

In contrast, the clutch size of females mated to the more attractive (three box) males did not change significantly with density. This is what we would predict if these females were already investing maximally in low density years because of their comparatively higher quality mate. Their clutch size would not increase with increasing density because they were already producing their maximum clutch size in low density years. Females who were lucky enough to have an apparently high quality mate for the current breeding attempt would have a very low chance of finding a better partner or breeding situation in the future no matter what the population density. Although they did not increase their clutch size during high density years, these females with attractive mates may have increased their investment in other components of nestling fitness such as allocation of nutrients to eggs, nestling provisioning, and nest defense. It is possible that the marginally non-significant trend toward decreasing clutch size during high density years among three box females was a result of these females shifting their reproductive investment from quantity to some aspect of quality during high density years. We did not consistently measure other forms of maternal investment across all of the years of our study, so we cannot directly address this issue. More research is needed investigating alternative forms of reproductive investment for females in both treatment groups.

Although we have assumed that nest box number is a signal of male attractiveness, we did not directly test the relationship between male quality and nest box number because we wanted to randomize male quality between our treatments. It is possible, though unlikely, that female response to nest box number occurs because cavity availability on a male's territory is connected to some environmental factor entirely unrelated to male quality. As long as cavity availability within a territory reflects the value of a particular breeding attempt in some way, female investment should vary with cavity availability regardless of whether it is related to male quality. However, we believe it is unlikely that cavity number is completely unrelated to male quality in nature because of the intense competition between male house wrens for territories (Kendeigh 1941; Johnson 1998) and the fitness benefits that multiple cavities provide for males (Soukup and Thompson 1998). We consider cavity availability an indirect measure of male attractiveness because in nature higher quality males should be able to obtain territories with more cavities (Dubois 2004; Eckerle and Thompson 2006). A study comparing characteristics of males that are allowed to settle naturally on territories containing various numbers of nest boxes prior to male arrival would test the appropriateness of this assumption.

Female response to nest box number, which we assume results from differential allocation based on male attractiveness, declined over time in this population. Further, this decline seems to be related to population density. What first appeared to be random noise and inconsistency in female behavior was actually readily explained by a population-level parameter. Whether density has widespread effects on sexually selected behaviors remains an open and interesting question. New studies that manipulate both density and male quality in a variety of systems would greatly advance our understanding of these processes.

REFERENCES

Afonso S, Vanore G, and Batlle A. 1999. Protoporphyrin IX and oxidative stress. Free Radical Research 31: 161-170.

Alworth T and Scheiber IBR. 2000. Nest building in House Wrens (*Troglodytes aedon*): a reexamination of male and female roles. Journal of Field Ornithology 71: 409-414.

Amundsen T. 2000. Why are female birds ornamented?. Trends in Ecology and Evolution 15: 149-155.

Ardia DR. 2007. Site- and sex-level differences in adult feeding behaviour and its consequences to offspring quality in tree swallows (*Tachycineta bicolor*) following brood-size manipulation. Canadian Journal of Zoology-Revue Canadienne de Zoologie 85: 847-854.

Aviles JM, Stokke BG, Moksnes A, Roskaft E, and Moller AP. 2007. Environmental conditions influence egg color of reed warblers Acrocephalus scirpaceus and their parasite, the common cuckoo Cuculus canorus. Behavioral Ecology and Sociobiology 61: 475–485.

Baird T, Solomon SE, and Tedstone DR. 1975. Localisation and characterisation of egg shell porphyrins in several avian species. British Poultry Science 16: 201-208.

Barnston AG and Livezey ER. 1987. Classification, seasonality, and persistence of low-frequency atmospheric circulation patterns. Monthly Weather Review 115: 1083-1126.

Bart J and Tornes A. 1989. Importance of monogamous male birds in determining reproductive success. Evidence for house wrens and a review of male-removal studies. Behavioral Ecology and Sociobiology 24: 109-116.

Both C. 1998. Density dependence of clutch size: habitat heterogeneity or individual adjustment?. Journal of Animal Ecology 67: 659-666.

Brylawski AM and Whittingham LA. 2004. An experimental study of mate guarding and paternity in house wrens. Animal Behaviour 68: 1417-1424.

Burley N. 1986. Sexual selection for aesthetic traits in species with biparental care. American Naturalist 127: 415-445.

Burley N. 1988. The differential-allocation hypothesis: an experimental test. American Naturalist 132: 611-628.

Burley RW and Vadhere DV. 1989. The Avian Egg. New York: John Wiley.

Cherry MI and Bennett ATD. 2001. Egg colour matching in an African cuckoo, as revealed by ultraviolet visible reflectance. Proceedings of the Royal Society of London Series B 268: 565-571.

Clutton-Brock TH. 1991. The Evolution of Parental Care. Princeton: Princeton University Press.

Cooper SW and Ritchison G. 2005. Nestling provisioning by male and female yellow-breasted chats: no relationships between morphology and parental care. Journal of Field Ornithology 76: 298-302.

Courrol LC, Silva FRD, Coutinho EL, Piccoli MF, Mansano RD, Vieira ND, Schor N, and Bellini MH. 2007. Study of blood porphyrin spectral profile for diagnosis of tumor progression. Journal of Fluorescence 17: 289-292.

Cunningham EJA and Russel AF. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404: 74-77.

Cuthill IC, Bennett ATD, Partridge JC, and Maier EJ. 1999. Plumage reflectance and the objective assessment of avian sexual dichromatism. American Naturalist 153: 183-200.

de Lope F and Moller AP. 1993. Female reproductive effort depends on the degree of ornamentation of their mates. Evolution 47: 1152-1160.

Dubois NS. 2004. Sexual selection and reproductive investment in house wrens (*Troglodytes aedon*). Ph. D. dissertation, Michigan State University.

Dubois NS, Kennedy ED, and Getty T. 2006. Surplus nest boxes and the potential for polygyny affect clutch size and offspring sex ratio in house wrens. Proceedings of the Royal Society of London Series B 273: 1751-1757.

Eckerle KP and Thompson CF. 2006. Mate choice in house wrens: nest cavities trump male characteristics. Behaviour 143: 253-271.

Eising CM and Groothuis TGG. 2003. Yolk androgens and begging behaviour in black-headed gull chicks: an experimental field study. Animal Behaviour 66: 1027-1034.

Endler JA. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41: 315–352.

Fisher RA. 1958. The Genetical Theory of Natural Selection (2nd ed.). Dover: New York.

Ferree ED. 2007. White tail plumage and brood sex ratio in dark-eyed juncos (*Junco hyemalis thurberi*). Behavioral Ecology and Sociobiology 62: 109-117.

Freeman-Gallant CR. 1996. DNA fingerprinting reveals female preference for male parental care in Savannah Sparrows. Proceedings of the Royal Society of London Series B 263: 157-160.

Getty T. 2006. Sexually selected signals are not similar to sports handicaps. Trends in Ecology and Evolution 21: 83-88.

Gil D, Graves J, Hazon N, and Wells A. 1999. Male attractiveness and differential testosterone investment in zebra finch eggs. Science 286: 126-128.

Gosler AG, Higham JP, and Reynolds SJ. 2005. Why are birds' eggs speckled? Ecology Letters 8: 1105–1113.

Hargitai R, Prechl J, and Torok J. 2006. Maternal immunoglobulin concentration in Collared Flycatcher (*Ficedula albicollis*) eggs in relation to parental quality and laying order. Functional Ecology 20: 829-838.

Head ML, Hunt J, and Brooks R. 2006. Genetic association between male attractiveness and female differential allocation. Biology Letters 2: 341-344.

Helfenstein F, Berthouly A, Tanner M, Karadas F, and Richner H. 2008. Nestling begging intensity and parental effort in relation to prelaying carotenoid availability. Behavioral Ecology 19: 108-115.

Heubel KU, Lindstrom K, and Kokko H. 2008. Females increase current reproductive effort when future access to males is uncertain. Biology Letters 4: 224-227.

Hinde CA and Kilner RM. 2007. Negotiations within the family over the supply of parental care. Proceedings of the Royal Society of London Series B 274: 53-60.

Jagannath A, Shore RF, Walker LA, Ferns PN, and Gosler AG. 2008. Eggshell pigmentation indicates pesticide contamination. Journal of Applied Ecology 45:133-140.

Jakob EM, Marshall SD, and Uetz GW. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77: 61-67.

Johnsen A, Delhey K, Schlicht E, Peters A, and Kempenaers B. 2005. Male sexual attractiveness and parental effort in blue tits: a test of the differential allocation hypothesis. Animal Behaviour 70: 877-888.

Johnson LS. 1998. House wren (*Troglodytes aedon*). In: The birds of North America (Ed. by Poole A and Gill F), pp. 380. Philadelphia, PA: The Birds of North America, Inc.

Johnson LS, Merkle MS, and Kermott LH. 1992. Experimental evidence for importance of male parental care in monogamous House Wrens. Auk 109: 662-664.

Johnstone RA and Hinde CA. 2006. Negotiation over offspring care—how should parents respond to each other's efforts?. Behavioral Ecology 17: 818-827.

Jones J, Doran PJ, and Holmes RT. 2003. Climate and food synchronize regional forest bird abundances. Ecology 84: 3024-3032.

Kendeigh SC. 1941. Territorial and mating behavior of the house wren. Illinois Biological Monographs 18:1-120.

Kendeigh SC. 1952. Parental care and its evolution in birds. Illinois Biological Monographs 22: 1-356.

Kennedy GY and Vevers HG. 1976. A survey of avian eggshell pigments. Comparative Biochemistry and Physiology B 55: 117–123.

Ketterson ED and Nolan V. 1994. Male parental behavior in birds. Annual Review of Ecology and Systematics 25: 601-628.

Kilner RM. 2006. The evolution of egg colour and patterning in birds. Biological Reviews 81: 383–406.

Kilner RM, Noble DG, and Davies NB. 1999. Signals of need in parent-offspring communication and their exploitation by the cuckoo. Nature 397: 667-672.

Kokko H. 1998. Should advertising parental care be honest?. Proceedings of the Royal Society of London Series B 265: 1871-1878.

Kokko H, Brooks R, Jennions MD, and Morley J. 2003. The evolution of mate choice and mating biases. Proceedings of the Royal Society of London Series B 270: 653-664.

Kokko H and Rankin DJ. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philosophical Transactions of the Royal Society B 361: 319-334.

Krist M and Grim T. 2007. Are blue eggs a sexually selected signal of female collared flycatchers? A cross-fostering experiment. Behavioral Ecology and Sociobiology 61: 863–876.

Limbourg T, Mateman AC, Andersson S, and Lessells CM. 2004. Female blue tits adjust parental effort to manipulated male UV attractiveness. Proceedings of the Royal Society of London Series B 271: 1903-1908.

Loyau A, Saint Jalme M, Mauget R, and Sorci G. 2007. Male sexual attractiveness affects the investment of maternal resources into the eggs in peafowl (*Pavo cristatus*). Behavioral Ecology and Sociobiology 61: 1043-1052.

MacGregor NA and Cockburn A. 2002. Sex differences in parental response to begging nestlings in superb fairy-wrens. Animal Behaviour 65: 923-932.

Magrath, R. D. 1991. Nestling weight and juvenile survival in the blackbird, *Turdus merula.* Journal of Animal Ecology 60: 335-351.

Magrath MJL and Komdeur J. 2003. Is male care compromised by additional mating opportunity?. Trends in Ecology and Evolution 18: 424-430.

Mallord JW, Dolman PM, Brown A, and Sutherland WJ. 2007. Quantifying density dependence in a bird population using human disturbance. Oecologia. 153: 49-56.

Martinez-de la Puente J, Merino S, Moreno J, Tomas G., Morales J, Lobato E, Garcia-Fraile S, and Martinez J. 2007. Are eggshell spottiness and colour indicators of health and condition in blue tits *Cyanistes caeruleus*?. Journal of Avian Biology 38: 377-384.

McGraw KJ. 2005. The antioxidant function of many animal pigments: are there consistent health benefits of sexually selected colourants?. Animal Behaviour: 69: 757-764.

McNamara JM, Gasson CE, and Houston AI. 1999. Incorporating rules for responding into evolutionary games. Nature 401: 368-371.

Mock DW, Schwagmeyer PL, and Parker GA. 2005. Male house sparrows deliver more food to experimentally subsidized offspring. Animal Behaviour 70: 225-236.

Moller AP and Thornhill R. 1998. Male parental care, differential parental investment by females and sexual selection. Animal Behaviour 55: 1507-1515.

Morales J, Sanz JJ, and Moreno J. 2006. Egg colour reflects the amount of yolk maternal antibodies and fledging success in a songbird. Biology Letters 2: 334–336.

Moreno J and Osorno JL. 2003. Avian egg colour and sexual selection: does eggshell pigmentation reflect female condition and genetic quality?. Ecology Letters 6: 803–806.

Moreno J, Osorno JL, Morales J, Merino S, and Tomas G. 2004. Egg colouration and male parental effort in the pied flycatcher Ficedula hypoleuca. Journal of Avian Biology 35: 300–304.

Moreno J, Morales J, Lobato E, Merino S, Tomas G, and Martinez-de la Puente J. 2005. Evidence for the signaling function of egg color in the pied flycatcher *Ficedula hypoleuca*. Behavioral Ecology 16: 931–937.

Moreno J, Lobato E, Morales J, Merino S, Tomas G, Martinez-de la Puente J, Sanz JJ, Mateo R, and Soler JJ. 2006a. Experimental evidence that egg color indicates female condition at laying in a songbird. Behavioral Ecology 17: 651–655.

Moreno J, Morales J, Lobato E, Merino S, Tomas G, and Martinez-de la Puente J. 2006b. More colourful eggs induce a higher relative paternal investment in the pied flycatcher *Ficedula hypoleuca*: a cross-fostering experiment. Journal of Avian Biology 37: 555–560.

Nakagawa S, Ockendon N, Gillespie DOS, Hatchwell BJ, and Burke T. 2007. Does the badge status influence parental care and investment in house sparrows? An experimental test. Oecologia 153: 749-760.

Nott MP, Desante DF, Siegel RB, and Pyle P. 2002. Influences of the El Nino/Southern Oscillation and the North Atlantic Oscillation on avian productivity in forests of the Pacific Northwest of North America. Global Ecology and Biogeography 11: 333-342.

Odabasi AZ, Miles RD, Balaban MO, and Portier KM. 2007. Changes in brown eggshell color as the hen ages. Poultry Science 86: 356-363.

OlsonVA, Liker A, Freckleton RP, and Szekely T. 2008. Parental conflict in birds: comparative analyses of offspring development, ecology, and mating opportunities. Proceedings of the Royal Society of London Series B 275: 301-307.

Osorno JL, Morales JM, Moreno J, Merino S, Tomas G, and Vasquez RA. 2006. Evidence for differential maternal allocation to eggs in relation to manipulated male attractiveness in the pied flycatcher (*Ficedula hypoleuca*). Journal of Ornithology 147: 605-611.

Ottoson U, Backman J, and Smith HG. 1997. Begging affects parental effort in the pied flycatcher, *Ficedula hypoleuca*. Behavioral Ecology and Sociobiology 41: 381–384.

Perrins CM and McCleery RH. 1989. Laying dates and clutch size in the great tit. Wilson Bulletin 101: 236-253.

Pizzolon M, Rasotto MB, Mazzoldi C. 2008. Male lagoon gobies, *Knipowitschia panizzae*, prefer more ornamented to larger females. Behavioral Ecology and Sociobiology 62: 521-528.

Preault M, Chastel O, Cezilly F, and Faivre B. 2005. Male bill colour and age are associated with parental abilities and breeding performance in blackbirds. Behavioral Ecology and Sociobiology 58: 497-505.

Qvarnstrom A. 1997. Experimentally increased badge size increases male competition and reduces male parental care in the collared flycatcher. Proceedings of the Royal Society of London Series B 264: 1225-1231.

R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Roed KH, Holand O, Mysterud A, Tverdal A, Kumpula J, and Nieminen M. 2007. Male phenotypic quality influences offspring sex ratio in a polygynous ungulate. Proceedings of the Royal Society of London Series B 274: 727-733.

Rundle HD, Odeen A, and Mooers AO. 2007. An experimental test for indirect benefits in Drosophila melanogaster. BMC Evolutionary Biology 7: 36.

Sauer JR, Hines JE, and Fallon J. 2008. The North American Breeding Bird Survey, Results and Analysis 1966 - 2007. Version 5.15.2008. USGS Patuxent Wildlife Research Center, Laurel, MD

Schulte-Hostedde AI, Zinner B, Millar JS, and Hickling GJ. 2005. Restitution of mass-size residuals: Validating body condition indices. Ecology 86:155-163.

Schwabl H. 1996. Maternal testosterone in the egg enhances postnatal growth. Comparative Biochemistry and Physiology A 114: 271-276.

Schwagmeyer PL and Mock DW. 2008. Parental provisioning and offspring fitness: size matters. Animal Behaviour 75: 291-298.

Shan Y, Pepe J, Lu TH, Elbirt KK, and Lambrecht RW. 2000. Induction of the heme oxygenase-1 gene by metalloporphyrins. Archives of Biochemistry and Biophysics 380: 219-227.

Sheldon BC. 2000. Differential allocation: tests, mechanisms and implications. Trends in Ecology and Evolution 15: 397–402.

Siefferman L, Navara K, and Hill G. 2006. Egg coloration is correlated with female condition in eastern bluebirds (*Sialia sialis*). Behavioral Ecology and Sociobiology 59: 651–656.

Sinclair ARE. 1989. Population regulation in animals. In: Ecological Concepts (Ed. By Cherrett JM), pp. 197-241. Oxford, England: Blackwell Scientific Publications.

Skinner AMJ and Watt PJ. 2007. Strategic egg allocation in the zebra fish, *Danio rerio*. Behavioral Ecology 18: 905-909.

Slagsvold T, Dale S, and Lampe HM. 1999. Does female aggression prevent polygyny? An experiment with pied flycatchers (*Fidcedula hypoleuca*). Behavioral Ecology and Sociobiology. 45: 403-410.

Smith HG. 1995. Experimental demonstration of a trade-off between mate attraction and parental care. Proceedings of the Royal Society of London Series B 260: 45-51.

Soler JJ, Moreno J, Aviles JM, and Moller AP. 2005. Blue and green egg-color intensity is associated with parental effort and mating system in passerines: support for the sexual selection hypothesis. Evolution 59: 636–644.

Soler JJ, Navarro C, Contreras TP, Aviles JM, and Cuervo JJ. 2008. Sexually selected egg coloration in spotless starlings. American Naturalist 171: 183-194.

Soukup SS and Thompson CF. 1997. Social mating system affects the frequency of extra-pair paternity in house wrens. Animal Behaviour 54: 1089-1105.

Soukup SS and Thompson CF. 1998. Social mating system and reproductive success in house wrens. Behavioral Ecology 9: 43-48.

Stearns SC. 1989. Trade-offs in life history evolution. Functional Ecology 3: 259-268.

Stearns SC. 1992. The evolution of life histories. Oxford, England: Oxford University Press.

Styrsky JD, Eckerle KP, and Thompson CF. 1999. Fitness-related consequences of egg mass in nestling house wrens. Proceedings of the Royal Society of London Series B 266: 1253-1258.

Stocker R, Yamamaoto Y, McDonagh AF, Glazer AN, and Ames BN. 1987. Bilirubin is an antioxidant of possible physiological importance. Science 235: 1043-1046.

Trivers RL. 1972. Parental investment and sexual selection. In: Sexual selection and the descent of man 1871-1971 (Ed. by Campbell B), pp. 136-179. London: Heinemann.

Thompson CF and Flux JEC. 1991. Body mass, composition, and survival of nestlings and fledgling starlings (*Sturnus vulgaris*) at Belmont, New Zealand. New Zealand Journal of Ecology 15:41-47.

Thompson CF, Flux JEC and Tetzlaff VT. 1993. The heaviest nestlings are not necessarily the fattest nestlings. Journal of Field Ornithology 64: 426-432.

Thompson DWJ and Wallace JM. 2001. Regional climate impacts of the Northern Hemisphere annular mode. Science 293: 85-89.

Underwood TJ and Sealy SG. 2002. Adaptative significance of egg coloration. In: Avian incubation. Behaviour, environment and evolution. (Ed. by Deeming DC), pp. 280-298. Oxford, England: Oxford University Press.

von Engelhardt N, Carere C, Dijkstra C, and Groothuis TGG. 2006. Sex-specific effects of yolk testosterone on survival, begging, and growth of zebra finches. Proceedings of the Royal Society of London Series B 273: 65-70.

Wallace AR. 1889. Darwinism. London: Macmillan and Co.

Weatherhead PJ. 2005. Effects of climate variation on timing of nesting, reproductive success, and offspring sex ratios of red-winged blackbirds. Oecologia 144: 168-175.

Wedell N, Kvarnemo C, Lessells M, and Tregenza T. 2006. Sexual conflict and life histories. Animal Behaviour 71: 999-1011.

Wilkin TA, Garant D, Gosler AG, and Sheldon BC. 2006. Density effects on life-history traits in a wild population of the great tit *Parsus major*: analyses of long-term data with GIS techniques. Journal of Animal Ecology. 75: 604-615.

Williams TD. 1994. Intraspecific variation in egg size and egg composition in birds: effects on offspring fitness. Biological Reviews 68: 35-59.

With TK. 1973. Porphyrins in egg shells. Biochemical Journal 137: 597-598.