**THE EFFECT OF MALE PLUMAGE COLORATION ON PARENTAL EFFORT
IN EASTERN BLUEBIRDS** (*Sialia sialis*)

by

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THESIS ABSTRACT

In iteroparous species, there is tradeoff between current and future reproductive investment. Parents that invest more in current offspring will provide resources that give those offspring better chances of survival and reproductive success. If the current brood is of poor quality, parents may invest less and save energy for other processes, as well as future reproduction, maximizing lifetime reproductive success. The differential allocation hypothesis predicts that individuals will adjust their parental investment based on the quality of their mate. Females will invest more in offspring of attractive males than unattractive males. In response, attractive males will invest less in current reproduction and allocate more time and resources to other processes. In the field, I tested the differential allocation hypothesis in the socially monogamous Eastern Bluebird (*Sialia sialis*). I experimentally manipulated the brightness of the structural blue coloration of Eastern Bluebirds, which is a known condition-dependent trait in the species. The brightness of male birds was either enhanced using a violet marker or reduced using a black marker. I recorded nest visitation rates prior to and after marker application to test the effects of treatment on both female and male visitation rates. I then tested the effect of treatment on offspring growth rates.

I predicted that 1) females mated to brighter males would visit the nest more than females mated to duller males, 2) brighter males would visit the nest less than duller males, and 3) offspring of brighter males would grow at a faster rate than offspring of duller males. I found a

trend suggesting that females of brighter males provision offspring at a higher rate than females mated to duller males, but there was no significant difference between males of each group. Tarsus growth rate of chicks between the groups did not differ, but chicks of the bright treatment gained mass at a faster rate than chicks of the dull treatment. The significant effect of treatment on chick body mass supported the trend demonstrated in female provisioning. I suggest that differential allocation by female bluebirds has the potential to create a selective pressure on male plumage brightness through enhanced offspring growth rates.

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INTRODUCTION

Indicator models of sexual selection suggest that elaborate ornaments in animals evolve to signal phenotypic or genetic quality (Zahavi 1975; Andersson 1994). Such signals can be used in mate choice or aid in intrasexual competition. In species with biparental care, these signals can indicate the quality of a variety of characteristics that could benefit potential mates, such as genetic quality, parental ability, physiological condition and resistance to stressors, parasite resistance, and competitive ability (Hill 2011). Honest signaling can evolve as a consequence of indirect or direct benefits of mate choice (Andersson 1994). Indirect benefit models suggest that there is a positive genetic link between an ornament and fitness (Kokko et al. 2002) and that heritably of the sexually selected trait is high (Kirkpatrick and Barton 1997). In contrast, direct benefits of sexual selection are more important when heritability of the trait is low (Kokko 1998). In birds, the most important direct benefits are typically the level of parental care (Heywood 1989).

The Good Parent Hypothesis, suggests that sexually selected ornaments signal parental quality (Hoelzer 1989). Under this hypothesis, individuals choose mates based on traits that signal potential parental quality. Several studies of bird coloration have supported the Good Parent Hypothesis (Senar et al. 2002; Maguire and Safran 2010). Males with more brightly colored feathers have been shown to provision offspring more frequently, acquire limited nest sites more successfully, and produce heavier offspring (Siefferman and Hill 2003; Siefferman and Hill 2005a; Grindstaff et al. 2012). Another hypothesis addressing parental care, specifically in species with biparental care is the differential allocation hypothesis. This hypothesis predicts

that individuals adjust their own parental investment based on the quality of their mate; mates of higher quality individuals are expected to provide more parental care than mates of less quality individuals, regardless of that individual's contributions (Burley 1986).

A second part of the hypothesis states that higher quality mates will reduce parental investment to save energy for future reproductive attempts, as well as increase its chance of survival, thus leading to directional selection on ornamental traits (Burley 1986). Parents are constantly faced with decisions related to the trade-off between current and future reproduction (Trivers 1972). Reproduction, as well as behaviors associated with parental investment, are a great energetic burden that affects the condition and ultimately the survival of individuals (Trivers 1972). Siefferman and Hill (2005) supported this hypothesis when they observed that male Eastern bluebirds (*Sialia sialis*) trade future ornamentation for current reproductive investment. When broods were experimentally reduced, males invested less in parental care and the reduction had a significant positive effect plumage brightness the following year. The differential allocation hypothesis directly addresses Trivers (1972) description of the trade-off between current and future reproductive attempts. Efforts to increase the fitness of current offspring are expected to increase costs associated with parents' own future survival and reproduction. Therefore, it is in the parent's best interest to adjust parental investment to the apparent value of the current offspring in each breeding attempt.

In avian species, differential allocation can occur at various breeding stages. For example, females can alter their investment in the quantity or quality of eggs produced (Johnsen et al. 2005), or through differential parental effort (Burley 1988; Johnsen et al. 2005; Limbourg et al. 2013). If the current mate is perceived as of higher quality and the probability that future mates are of equal or higher quality is low, then current offspring will have a higher value than

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the expected value of future offspring. Parental care is costly (Clutton-Brock 1991), so selective investment must be balanced by a benefit (Moller and Thornhill 1998), such as more attractive and higher quality offspring.

I experimentally tested the differential allocation hypothesis in the field on a breeding population of Eastern bluebirds in Alabama. The Eastern bluebird is a socially monogamous, sexually dimorphic species that exhibits biparental care (Gowaty and Plissner 1998). Males display a structural UV-blue coloration on the rump, back, head, wings, and tail. Females display the same, but duller plumage pattern. The UV-blue plumage is produced through modification of the nanostructure of feather barbs (Shawkey et al. 2005; Shawkey et al. 2006). A feather barb has an outer keratin cortex, beneath which is a spongy layer of air spaces and keratin rods. The precise size and arrangement of those nanostructures allows for coherent scattering of light that allows short wavelengths of light, blue and violet colors, to be reflected and amplified, while longer wavelengths of light to get absorbed by melanin under the spongy layer. The blue plumage brightness of bluebirds has been shown to correlate with individual condition in both males and females (Siefferman and Hill 2005b; Siefferman and Hill 2005a; Siefferman et al. 2005; Grindstaff et al. 2012). Individual condition, particularly that associated with nutrition, is believed to affect structural coloration due to deficiencies in the precision of the formation of feather nanostructure (Hill 2006a). Structural coloration has also been shown to be a positive indicator of offspring provisioning (Keyser and Hill 2000; Siefferman and Hill 2003; Limbourg et al. 2004; Johnsen et al. 2005; Grindstaff et al. 2012).

Many previous studies have tested the differential allocation hypothesis by altering male phenotypic traits associated with quality, often by using artificial traits (Burley 1988; Swaddle 1996) or by manipulating outside the natural variation of an ornament (de Lope and Moller 1993;

Limbourg et al. 2004). A better approach is to manipulate the trait within the range of natural variation of male attractiveness (Hill 2006b), as in a study of male blue tits (*Cyanistes caeruleus*) (Johnsen et al. 2005). I altered male Eastern bluebird plumage coloration using either a black marker to decrease the brightness of the feathers making them appear duller or a violet marker to increase the brightness of the feathers and the perceived quality of the individual. It has previously been demonstrated that these methods can alter plumage color within its range of natural variation (Liu et al. 2007). I then quantified the change in rate of parental visitations for both treatment groups. In accordance with the differential allocation hypothesis, I predicted that (1) females paired to males with enhanced blue plumage brightness would have a higher nestling visitation rate than females paired with experimentally reduced blue plumage brightness, and also that, (2) males with enhanced blue plumage brightness would have a lower nestling visitation rate than males with reduced blue plumage brightness.

METHODS

Study Species.- The Eastern bluebird is a medium-sized songbird that breeds throughout eastern North America (Gowaty and Plissner 1998). Eastern Bluebirds are cavity-nesters and adults practice biparental care of offspring. Adult males have brilliant blue plumage on their heads, backs, rumps, tails, and wings, while their upper breast feathers are a warm orange-brown color and bellies white. This blue coloration has a spectral reflectance peak at approximately 400 nm and is known to reflect UV and blue wavelengths equally (Siefferman and Hill 2003). Adult female bluebirds are paler and blue-gray on top with dull blue wing and tail feathers and pale orange breasts.

Study Site and General Field Procedures.- The study was conducted in early April through July of 2012. Two groups of nestboxes were monitored (approximately 11.3 km apart) in Auburn, Lee County, Alabama. Bluebirds at these locations have been banded since 1999. Any adult birds that were not previously banded were banded upon capture with a U.S. Fish and Wildlife aluminum band and a unique set of 3 color bands. All pairs of bluebirds included in the study were rearing their second clutch that year. The first day of hatching for a nestbox was classified as day 1. I caught adult males attending chicks on day 5 post-hatching for color manipulation. Males were mist netted between the 1000 and 1200 h, and a second attempt was made at 1700 and 1900 h, as necessary. Nest visitation rate was recorded 3 hr on days 7 and 10 starting at sunrise (See Fig. 1 for timeline). I used Eastern Bluebird playback calls and a male bluebird museum skin to attract males to the net. I carefully plucked 7-10 rump feathers from each bird. All feathers were placed in sealed envelopes for future spectrometry analysis.

Previous studies have demonstrated that permanent markers can be used to experimentally enhance or reduce structural plumage brightness within the natural range of variation, while not eliminating UV reflectance (Ballentine and Hill 2003; Liu et al. 2007). Plumage brightness has been shown to be condition dependent in the Eastern Bluebird, while hue and chroma have not, therefore brightness is what was manipulated in this experiment (Siefferman and Hill 2005a). Because structural coloration is produced by feather nanostructure, rather than pigments, feathers colored with markers still looked blue to a human observer (Liu et al. 2007). I used non-toxic, permanent markers (Sharpie ® permanent marker) to either decrease brightness of the natural plumage with a black marker or increase brightness with a violet marker. I carefully colored all feathers on the head, back, rump, tail, and wings of each bird. To confirm that reflectance spectra of the manipulated feathers reflected natural variation in color

within the population (as shown by Liu et al. 2007), feathers were collected from male birds outside the study for post-manipulation samples. After color application, the bird was placed in a brown paper bag 1-2 minutes to allow the color to dry, and then birds were released. Nestboxes were assigned to a treatment group randomly for each location.

Visitation Rate.- All nestboxes where a male was successfully captured and color manipulated were monitored for parental visitation. Birds were first monitored with cameras on day 5, prior to color manipulation to determine initial visitation rate. On days 7 and 10, activity at nestboxes was recorded with a Reconyx game camera for 3 hr, starting at sunrise. Pictures were taken at a rate of one per second and thus, making it unlikely that visitations were missed. The sex of the parents feeding the chick was determined based on characteristic plumage. If I could not consistently determine the difference between the male and female of a particular box, that box was not used in the study. Parental visitation rate was quantified rather than feeding rate because I could not be confident the male or female brought food to the nest with each visit. A visitation attempt was counted when the adult inserted its full head into the nestbox. The treatment group that each male belonged to was blind to the observer quantifying visitations at the nest avoid biases. The number of visitations was recorded for males and females on day 5 prior to treatment, and then 2 days and 5 days post-treatment, which was equal to day 7 and 10 post-hatching. Relative visitation rate was quantified relative to the visitation rate prior to the color manipulation, as the number of visits on day 7 divided by the number of visits on day 5, and number of visits on day 10 over the number of visits on day 5

Spectrometry Analysis.- The rump feathers collected from the adult males were taped in an overlapping pattern to recreate a colored patch and then along with the tail feathers taped onto black construction paper. Plumage reflectance was measured using an Ocean Optics S2000

spectrometer and deuterium tungsten halogen light source (Dunedin, Florida, USA) and a micron fiber-optic probe with a rubber cap (to exclude ambient light) held at a 90° angle to the feather surface. I took five measurements for each feather sample and averaged them to represent the UV-blue coloration of each male. The program CLR (v1.05) was used for spectral analysis. I produced spectral curves for normal and manipulated feathers to show how the manipulation affected the percent reflectance. CLR calculates mean brightness as the average reflectance in the 300-700 nm range.

Statistical Analysis.- All analyses were performed with JMP 10.0.2 (SAS Institute, Cary, NC) and p-value of ≤ 0.05 was considered statistically significant. To determine if the treatment groups had equal variance, clutch size, pre-treatment nest visitation rate, and male coloration pre-treatment were compared between groups using a two-sample t-test. . A t test was also used to show that there was a treatment effect on brightness of plumage from the markers. Controlling for the effects of repeated data within individual (day 7 and 10), a repeated measures analysis of variance (ANOVA) was used to compare the relative visitation rate between treatment groups at day 7 and day 10. Nest visitation rates were transformed prior to analysis.

RESULTS

Clutch Size and Pre-Treatment Variables.- There was no difference in clutch size between treatments (t= 0, df= 17.6, P= 1.0). The number of times males (t= 0.63, df= 13, P= 0.537) and females (t= 1.45, df= 12.79, P= 0.173) visited the nest did not vary between groups prior to treatment application. Pre-treatment male plumage brightness on day 5 did not vary between groups (t= -0.92, df= 6.05, P=0.395).

Spectrometry.- Both treatments produced spectral curves characteristic of blue (Fig. 2). Mean brightness for the feathers of all groups fell within the natural range of variation as reported by Liu et al. (2007). The bright treatment had a mean brightness significantly higher than the dull treatment (t= -4.79, df= 5.03, P= 0.005*).*

Female Visitation Rate.- The overall model testing for the effect of treatment, days post-manipulation, and individual ID on female relative visitation rate bordered on significance (overall: $F_{15, 8}$=2.93, P=0.065), with females paired to more brightly colored males increasing their number of visitations relatively more than females paired to dull colored males (treatment effect: partial F_1= 15.08 P= 0.005) (Fig. 3). Relative provisioning rate did not vary between day 7 and 10 (repeatability effect: partial F_1= 4.97, P= 0.056) and visitation rate did not vary between individual females (ID effect: partial F_{13}= 1.67, P= 0.238).

Male Visitation Rate.- The overall model testing for the effects of treatment, days post-manipulation, and individual on male relative visitation rate was significant (overall: $F_{15,8}$= 4.18, P= 0.024), but this effect was driven by a significant variation in visitation rate between individual males (ID effect: partial F_{13}= 4.10, p= 0.026). Male plumage brightness (treatment effect: partial F_1= 3.63, P= 0.093) and relative provisioning rate between day 7 and 10 (repeatability effect: partial F_1= 0.06, P= 0.807) was not significant (Fig. 4).

DISCUSSION

Burley's (1986) differential allocation hypothesis predicts that because there is a trade-off between current and future reproduction, mates of higher quality individuals will invest more in the current offspring than mates of lower quality individuals. The results of this study are suggestive that differential allocation may exist in Eastern Bluebirds. There was a trend

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suggesting that females mated to brighter males increase their visitation rate to a greater degree as chicks age that females mated to duller males, suggesting that female may interpret their mates as in better or worse condition, respectively. Similar results were found in other studies testing the differential allocation hypothesis. In blue tits, Johnsen et al. (2005) found male color typically does not impact female provisioning rates, with the exception of first time breeders (1-year olds) that did display differential allocation. In a separate study, Limbourg et al. (2004) showed that blue tit females decreased their feeding rates when males displayed reduced coloration.

Visitation rate was used as a measure of parental effort because it could not be reliably determined whether parents carried food upon entering the nestbox. Nevertheless, the behaviors displayed at each visit were suggestive of feeder and thus, feeding and nest visitation should be strongly correlated. As nestlings age and grow they require greater quantities of food, thus parental visitation rate to increase through nestling development. Although the change in visitation rate between day 5 and 7 and 5 and 10 average greater than 1 across all treatment groups, there was no difference in provisioning rates between days 7 and 10. It is possible that no low sample size contributed to lack of difference between these two time points.

Under the differential allocation hypothesis, mate quality is used as a tool for making decisions between current and future reproduction. Females may be more likely to express positive different allocation earlier in a breeding season, or earlier in a lifetime, when there is a higher possibility of more breeding attempts. It is in best interest for individuals to maximize their investment in the best quality mates. This may explain why only young females significantly expressed positive differential allocation in Limbourg et al. 2004. Unfortunately, the sample size was not large enough in this study to test for this effect.

In Burley's (1986) original presentation of the differential allocation hypothesis, it is stated that individuals may increase their parental investment to either obtain or maintain their high quality mates. Another interpretation for the trend of more maternal investment displayed by females when mated to high quality mates is that they are trying to maintain their mate for future reproduction. If females mated to brighter males display increased parental effort, then males are expected to decrease their own parental investment in response to save energy for future reproduction or extra-pair mating attempts. Moller and Thornhill (1998) showed that differential allocation strategies are associated with higher frequencies of extra-pair paternity, and it has been specifically demonstrated in the closely related Mountain Bluebird (*Sialia currucoides*) that brighter blue males sired more offspring both with their own mate and extra-pair mates than duller males (Balenger et al. 2009). It is possible that higher quality males have the chance to breed with higher quality females in subsequent breeding attempts. Then, if a female recognizes that her mate is in good condition and can provide benefits to her offspring, she may increase her parental investment to retain her mate for future reproduction.

Although males cannot judge their own altered phenotype, it is most likely that they receive cues from the female about their phenotypic condition (Johnsen et al. 2005). These cues could be transferred through brief interactions made with their mate during the nestling period or could be cues from the increased parental effort by the females. Burley (1986) predicted that high quality mates would decrease their parental investment as compensation for the increase parental investment in their mates. She later found support for this claim in zebra finches (*Taeniopygia guttata*) where the male's phenotypic was manipulated with color bands (Burley 1988). "Attractive" males decreased their parental investment as females increased their parental investment. A similar result was observed in the study done by Johnsen et al. (2005) on blue tits.

UV reduced (less attractive) males fed at a higher rate than UV enhanced (more attractive) males. My study demonstrated that male Eastern Bluebirds that were experimentally made brighter showed no difference in nest visitation from males that were dulled, which does not support the second prediction made by the differential allocation hypothesis. This finding agrees with a similar study done on blue tits where they manipulated male attractiveness by eliminating UV reflectance in a group of males (Limbourg et al. 2004). However, it disagrees with what has been observed in other studies of differential allocation on birds. Differences between this study and other studies may be an effect of treatment time period; males may respond differently based on the time of the nestling stage that manipulation occurs.

This study demonstrates support for primary predictions of the differential allocation hypothesis; individuals paired to brighter mates, have higher parental investment than individuals paired to duller mates. Female bluebirds showed a trend for a greater change in nest visitation rate when paired with brighter males. However, my observations did not support the second assumption made by the differential allocation hypothesis that high quality mates will have reduced parental investment to save for future reproduction. Females appear to be constantly accessing the condition of their mate to make decisions regarding the tradeoff between current and future reproduction. The level of parental effort expressed by female bluebirds has the ability to create directional selection pressures on male structural coloration.
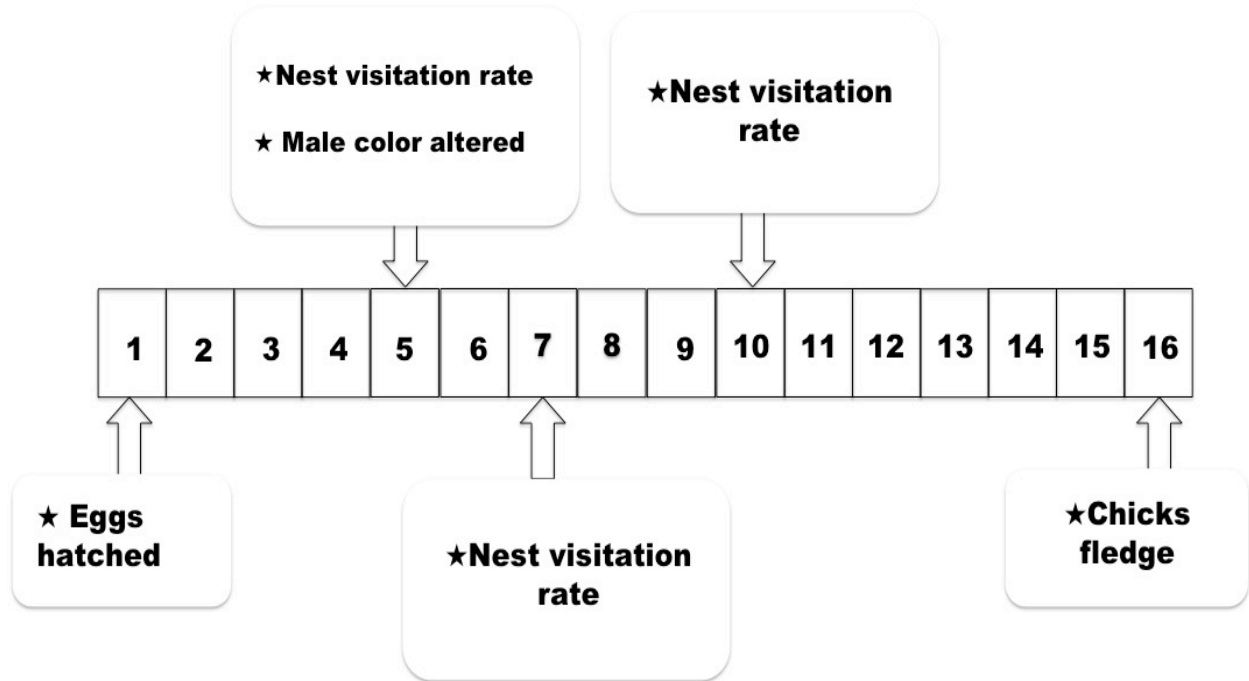
Figure 1. A timeline of nesting sequence and procedures in the study. Numbers in timeline represent days from hatching of eggs.

Figure 2. Reflectance spectra of male plumage coloration showing the effect of treatment by permanent markers on rump feathers. The *think black line* represents the natural male plumage coloration before treatment. The *gray line* represents the brightness-enhanced treatment by violet markers. The *thick black line* represents the spectral curve of the brightness-reduced treatment by black markers.

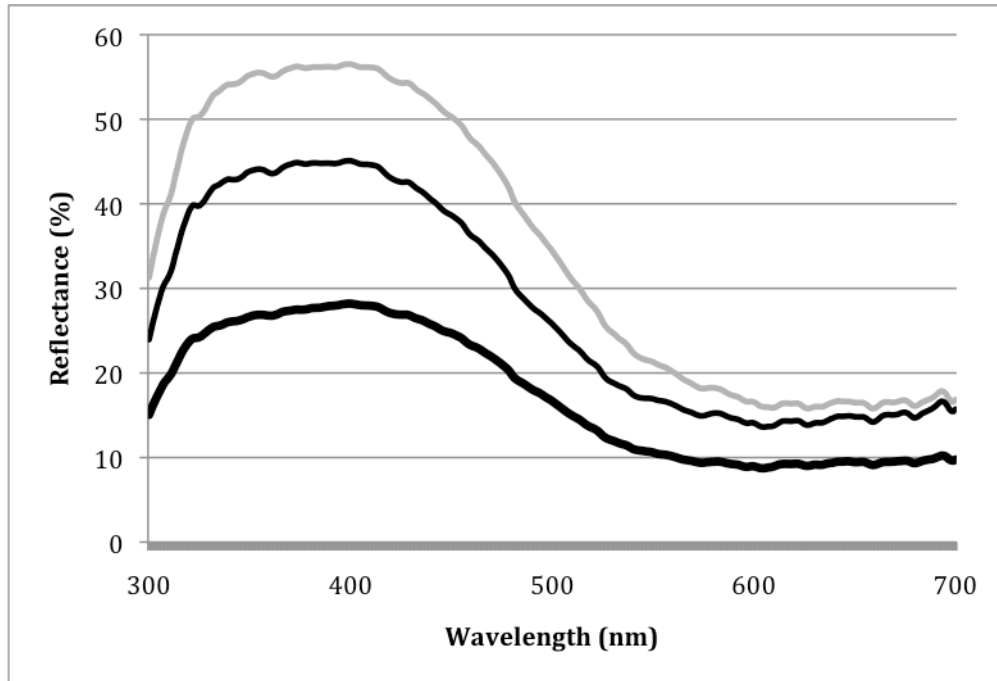Figure 3. Change in the rate at which female Eastern Bluebirds visited nestlings following experimental manipulation of the color of the male at days 7 and 10. Dark columns represent females paired with males of enhanced plumage brightness. Light columns indicate females paired with males of reduced plumage brightness. Visitation rate is presented relative to the rate of visitation before manipulation on day 5.
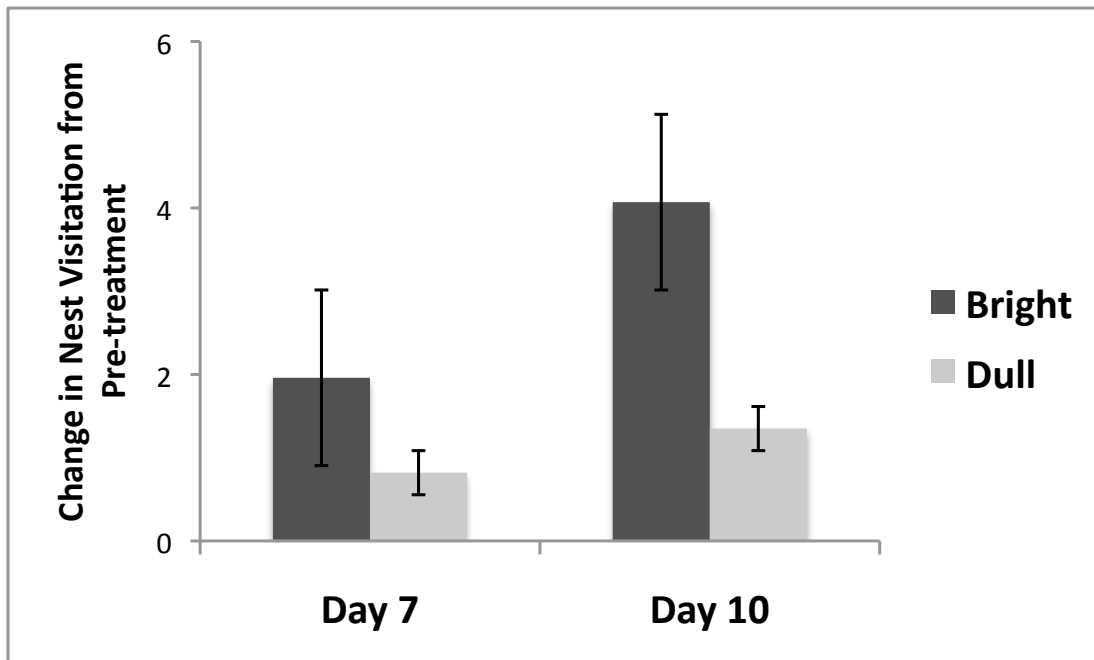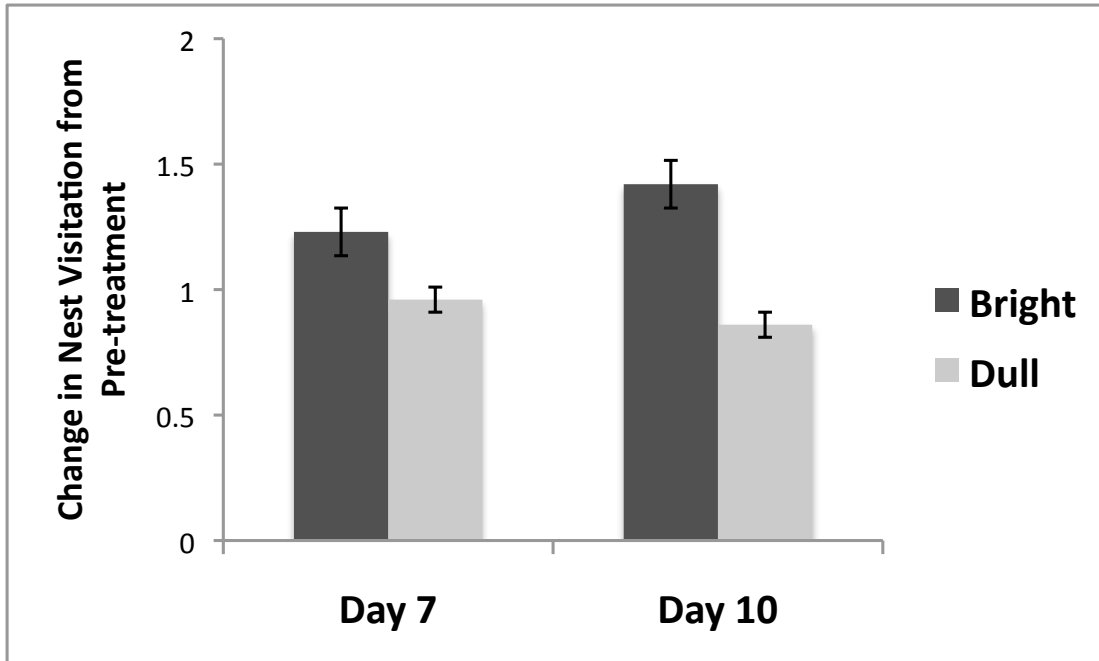
Figure 4. Change in the rate at which male Eastern Bluebirds visited nestlings following experimental manipulation of the color of the male at days 7 and 10. Dark columns represent males with enhanced plumage brightness. Light columns indicate males with reduced plumage brightness. Visitation rate is presented relative to the rate of visitation before manipulation on day 5.

CAN ALTERED MALE PHENOTYPE AFFECT OFFSPRING GROWTH

RATES?

INTRODUCTION

In species with repeated reproduction, a tradeoff many occur between current and future

reproductive effort (Trivers 1972). This conflict arises because, while parental investment is

beneficial for viable offspring, it presents a substantial cost in terms of reduced survival or

fecundity to the parent (Clutton-Brock 1991). Species with biparental care deal with an added

element to the conflict, because there is often a negotiation between both sexes over the level of

investment (Hinde 2006). In birds and other species with ornamental displays, the relative level

of parental investment is often correlated with the quality of ornamental traits (e.g. refs). Theses

traits can be both exaggerated and costly and may even adversely affect survival (Darwin 1871).

Ornamental traits are typically associated with honest signals of genetic quality (Zahavi 1975).

But Hoelzer (1989) suggested by the Good Parent Hypothesis that ornamental traits could be

sexually selected as indicators of potential parental ability. In species where parental ability is

correlated with the execution of a trait, it is in the best interest of one individual to mate with

another with a higher quality display that will not only provide more parental care, but will pass

along genes associated with a higher quality display. The ability to acquire good nesting sites,

provide nest defense, and feed nestlings and mates are examples of direct benefits provided by

good parents (Heywood 1989; Hoelzer 1989). Traits associated with parental ability are often

used in negotiations between parents in relative level of care (Hinde 2006), as well as in

tradeoffs between current and future breeding attempts (Burley 1986).

If mate selection is driven by selection for parenting ability, as suggested by good parent

hypothesis, then it is also probably that relative parental effort may be adjusted based on the

quality of that mate (Burley 1986). The differential allocation hypothesis, as described in chapter 1, assumes that: 1) the potential for mates with high quality ornaments to produce higher quality offspring must be greater than mates with low quality ornaments, 2) resource investment by the choosing sex must be costly, creating a tradeoff between investment and condition, and 3) the resource must be beneficial to the offspring of the current reproductive attempt (Sheldon 2000). Most often it is the female that is differentially allocating parental investment based on the attractiveness of the male. It is assumed that females of most species have a finite, renewable amount of resources to use for reproduction (Harris and Uller 2009). Thus, by the differential allocation hypothesis, females are expected to invest more in the most viable offspring. Females that invest more in a current reproductive attempt will be at a selective disadvantage unless their investment is balanced, or preferably outweighed, by a benefit (Moller and Thornhill 1998). By investing more in the offspring of mates with that indicate high genetic and phenotypic quality, a female will further increases the potential quality of the offspring. Females will obtain an indirect fitness benefit because their offspring should experience enhanced ornament quality ultimately resulting in a greater long-term fitness payoff (Moller and Thornhill 1998).

Several studies across different taxa have demonstrated support for the differential allocation hypothesis (reviewed in Sheldon 2000). Most of these studies have focused on the direct benefits associated with sexual selection, such as parental effort, nest defense, and clutch size. However, few studies testing differential allocation have focused on the reproductive output and success of offspring (Sheldon 2000). For the cost of differentially allocating resources to current offspring to be beneficial, enhanced offspring viability must exist (Burley 1986). Reproduction and parental care are costly endeavors, and those that invest more usually suffer decreases in survival and condition in following years (Clutton-Brock 1991). If females invest

more in the current brood, without experiencing increased reproductive success, than they are at a selective disadvantage (Moller and Thornhill 1998). Therefore, there is a need to not only to study parental investment as it relates to the differential allocation hypothesis, but also to see if parental investment influences indirect genetic effects for the offspring.

As a consequence to the differential allocation hypothesis, the higher investment in offspring of attractive mates should lead to increased offspring condition (Limbourg et al. 2004). Not only direct genetic effects, but also indirect genetic effects, of parental quality influence offspring viability (Clutton-Brock 1991). Indirect genetic effects can occur whenever individuals provide non-genetic resources to offspring (Wolf et al. 1997). Wolf et al. (1997) also refers to these types of effects as "inherited environments", because while they are effects due to the environment in the offspring generation, the phenotypes of the parental generation that produced the environmental effects are heritable. Thus, if an individual differentially allocates resources based on mate attractiveness or quality, it can create a link between mate attractiveness and offspring condition (Harris and Uller 2009). Without an increase in offspring condition and survival, a parent's effort is in vain.

The Eastern Bluebird (*Sialia sialias*) is a well-studied organism for its plumage coloration in relation to aspects of sexual selection and parental care. It is a socially monogamous, sexually dimorphic species with biparental care of offspring (Gowaty and Plissner 1998). Males display a brilliant ultraviolet (UV) blue structural coloration on the rump, back, head, wings, and tail. Females display a similar, but duller blue-grey pattern. The UV-blue structural coloration is due to changes in the nanostructure of the feathers (Shawkey et al. 2006). The nanostructure, which affects the quality of the structural coloration, is thought to be greatly affected by factors associated with individual condition (Hill 2006). The blue plumage of Eastern

Bluebirds is considered an ornamental trait that has experimentally been shown to correlate with factors associated with condition in both sexes (Siefferman and Hill 2005a; Siefferman and Hill 2005b; Siefferman et al. 2005; Grindstaff et al. 2012). Structural coloration is also known to be associated with parental ability in many species, including in Eastern Bluebirds (Keyser and Hill 2000; Siefferman and Hill 2003; Limbourg et al. 2004; Johnsen et al. 2005; Grindstaff et al. 2012). It has previously been demonstrated that more colorful bluebirds produce heavier offspring (Siefferman and Hill 2003; Grindstaff et al. 2012). Female bluebirds may use the blue plumage in males as an indicator of the value of potential offspring (Limbourg et al. 2004), and differentially allocate more resources to brighter blue males. If unattractive males receive decreased provisioning from females, their offspring will experience hindered development of sexually attractive traits, as well as decreased body size (Limbourg et al. 2004). Small body size in offspring is associated with adverse effects on survival post fledging and competitive ability later in life (Garnett 1981; Alatalo and Lundberg 1986).

In this study I experimentally manipulated male Eastern Bluebird attractiveness by altering blue plumage brightness. Natural variation of structural plumage is due to coherent scattering of light from the spongy layer of feather barbs (Shawkey et al. 2006). I was able to change male coloration to the extremes of the blue spectrum seen in nature using markers (protocol from Liu et al. 2007). Males were either made more attractive using a blue marker, or less attractive using a black marker. I tested whether females observed the change in phenotype, and invested more in "higher quality" males as seen by altered offspring condition. Offspring condition was quantified as the change in clutch growth rate. In Eastern Bluebirds, females that invest more in current reproduction are less likely to survive to the following year (Siefferman and Hill 2008). If the current mate is considered attractive, females are expected to provide more

care and produce offspring of better quality, trading off their own survival and future

reproductive success. I expect offspring of experimentally enhanced male color to be of better

condition than offspring of experimentally reduced male color in Eastern Bluebirds.

METHODS

Study Site and General Field Procedures.- As described in chapter 1, monitoring of

bluebird nesting began in early April and continued through July of 2012. All data collected in

this study was limited to the second clutch of each bluebird pairs. The day that the first egg of a

clutch hatched was considered day 1. The to color manipulation was applied to males on day 5,

as described in chapter 1. Following color manipulation, the chicks were removed from the nest

and body mass and tarsus length was recorded for all chicks. Chick morphological measurements

were recorded again on days 7 and 10 between the 1000 and 1200 hr (See Fig. 1 for timeline of

study) body mass was measured to the nearest 0.01 g using a digital balance. Tarsus length was

measured to the nearest 0.01 mm using calipers. Wing chord and bill length were also measured,

but were not used in analyses.

Color Manipulation.- It has been demonstrated that for Eastern Bluebirds plumage

brightness, and not chroma or hue, is a condition-dependent trait (Siefferman and Hill 2005a),

therefore brightness was the component of structural plumage that was manipulated in this study.

Permanent markers have been shown to be able to enhance or reduce structural plumage

brightness with the natural range of variation, while not eliminating UV reflectance (Ballentine

and Hill 2003; Liu et al. 2007). Because structural coloration is produced by feather

nanostructure, rather than pigments, feathers colored with markers still looked blue to a human

observer and color measurements still fall within the range of natural variation (Liu et al. 2007). I

used non-toxic, permanent markers (Sharpie ® permanent marker) to either decrease with a

black marker or increase with a violet marker the brightness of male natural plumage. Upon capture, I carefully colored all feathers on the head, back, rump, tail, and wings of each bird. Once color was applied, I placed the bird in a brown paper bag for 1-2 minutes to allow the color to dry, and then the birds were released near the nest. Nestboxes were assigned to treatment groups randomly for each location. Manipulated feathers were collected from male birds 2-3 days post-manipulation from separate nestboxes that were not a part of the study to confirm that reflectance spectra reflected natural variation in color within population, as shown previously by Liu et al. (2007).

Chick Morphological Measurements.- Pre-treatment morphological measurements were taken for all chicks in a brood on day 5, and post-treatment measurements were taken on days 7 and 10 morphological measurements were taken again between the hours of 1000 and 1200. Mass was measured to the nearest 0.01 g using a digital balance. Tarsus length was measured to the nearest 0.01 mm using calipers. Wing chord and bill length were also measured, but were not used in analyses.

Spectrometry Analysis.- The rump feathers collected from the adult males were taped in an overlapping pattern to recreate a colored patch and then along with the tail feathers taped onto black construction paper. Plumage reflectance was measured using an Ocean Optics S2000 spectrometer and deuterium tungsten halogen light source (Dunedin, Florida, USA) and a micron fiber-optic probe with a rubber cap (to exclude ambient light) held at a 90° angle to the feather surface. I took five measurements for each feather sample and averaged them to represent the UV-blue coloration of each male.

I used the program developed by Montgomerie (2008) (CLR v1.05) to analyze reflectance data. Percent reflectance data was used to create a spectral curve for natural and

manipulated feathers. Because brightness is known to be the trait associated with condition dependence in the species, it was the standard descriptor of reflectance that I used. Mean brightness is the average reflectance in the 300-700 nm range. Analysis was done on both rump and tail feathers, but both feathers displayed a similar pattern so all data reported is indicative of rump feathers.

Statistical Analysis.- All analyses were performed with JMP 10.0.2 (SAS Institute) and a p-value ≤ 0.05 was considered statistically significant. A two-sample t test was used to confirm that there was not a significant difference in clutch size, pre-treatment chick tarsus length, pre-treatment chick mass, and male coloration pre-treatment between groups. A t test was also used to demonstrate if the markers had an effect on brightness between groups. A repeated measures analysis of variance (ANOVA) was used to examine the effect of color manipulation on growth of chick tarsus length and chick mass. ID number was included as a covariate to control for repeated sampling events within individual (day 7 and 10). Chick age and the interaction between days and color manipulation were also included in the model. Tarsus length and chick mass were log transformed prior to analysis.

RESULTS

Clutch Size and Pre-Treatment Variables.- There was no difference in clutch size between treatments (t= 0, df= 17.6, P= 1.0). Pre-treatment male plumage brightness on day 5 did not vary between groups (t= -0.92, df= 6.05, P=0.395). Average clutch tarsus length (t= 1.17, df= 19.9, P= 0.255) and mass (t= 0.98, df= 19.9, P= 0.338) did not vary prior to treatment.

Color Analysis.- The manipulated brightness of the two treatment groups was shown to be significantly different from one another (*t=-4.78, df= 5.03, p= 0.005*), indicating that the permanent marker treatments effectively changed the plumage coloration of male Eastern

Bluebirds. The mean brightness for both treatment groups fell within the natural range of plumage brightness reported by Liu et al. (2007). The treatments appeared to affect plumage brightness but did not significantly alter the shape of the spectral curve (Fig. 2).

Change in Tarsus Length

The overall model testing for the effects of treatment, age, and individual on relative chick tarsus growth for a clutch was significant (overall: $F_{22,15}= 6.15$, P= 0.0004), but this effect was driven by a significant variation in tarsus growth between individual clutches (clutch ID effect: partial $F_{20}= 3.10$, p= 0.015 and the growth in the length of the tarsus between days 7 and 10 (age effect: partial $F_1= 49.6$, P< 0.0001). Clutch tarsus length between treatments (treatment effect: partial $F_1= 2.19$, P= 0.160) was not significantly different (Fig. 2).

Change in Body Mass

The overall model testing for the effect of treatment, days post-manipulation, and individual ID on change in body mass change was significant (overall: $F_{22,15}= 4.86$, P=0.0014), with clutches of brightly colored males having a faster rate of mass change than clutches of dull colored males (treatment effect: partial $F_1= 4.71$, P= 0.046) (Fig. 3). Change in clutch mass varied between days 7 and 10 significantly (age: partial $F_1= 56.7$, P< 0.0001). Clutch mass change did not vary between clutches (clutch ID effect: partial $F_{20} = 1.75$, P= 0.135).

DISCUSSION

In this study, I tested the effect of male ornamentation on offspring growth rate by experimentally manipulating the plumage coloration of males. I found no difference in rate of tarsus growth between clutches of treatments. However, I did find a significant effect of treatment on mass change. There was a significant difference in plumage coloration due to manipulation between the bright and dull treatments. I previously demonstrated that females in

pairs where the male was experimentally brightened showed a trend for visiting the nest at a higher rate than females paired to males with reduced brightness (Chapter 1). Interestingly, even over just a period of a few days, this trend in females appears to have an effect on nestling growth. Nestling body size is genetically determined, but can be influenced by environmental effects (Senar et al. 2003). It has been demonstrated that brighter male and female plumage in Eastern Bluebirds is associated with heavier offspring or offspring in better condition (Siefferman and Hill 2003; Siefferman and Hill 2005a; Grindstaff et al. 2012). In a study using blue tits (*Cyanistes caeruleus*) where male phenotype was experimentally reduced offspring had lower growth rates (Limbourg et al. 2004). However, previous studies looking at the effect of male phenotype on offspring fitness have found no effect (Sheldon 2000; Johnsen et al. 2005; Balenger et al. 2007). Differences between studies could be species-specific or come from different time points when measurements were taken.

Increased investment in offspring of attractive mates should lead to increased offspring condition (Burley 1986; Sheldon 2000). In my first chapter I found that Eastern Bluebird females mated to brighter males showed a trend towards a higher relative provisioning rate than females mated to duller males. The only way for the trade-off proposed by Trivers (1972) between current and future reproduction to be beneficial for the parent is for the costs of reproduction to be outweighed by the benefits of enhanced offspring viability. It was experimentally demonstrated in a study of *Drospholia melanogaster* that the direct cost of differentially allocating more resources to offspring of attractive mates was outweighed by the indirect benefits of offspring quality (Head et al. 2005). Small body size of nestlings is known to have adverse effects on survival post fledging and competitive ability later in life (Garnett 1981; Alatalo and Lundberg 1986). Decreased provisioning is suggested to also hamper development

of sexually attractive traits (Ohlsson et al. 2002; Blount et al. 2003). To demonstrate the possible benefit of increased reproductive effort for offspring of attractive mates it would have been useful to test for future survival, sexual attractiveness, and reproductive success of the offspring. A better measure of reproductive success and offspring quality may be necessary to better understand the benefit of enhanced parental effort related to attractive mates. However, it is interesting that a change in male phenotype has the ability to effect offspring condition through female behavior over such a short span of time.
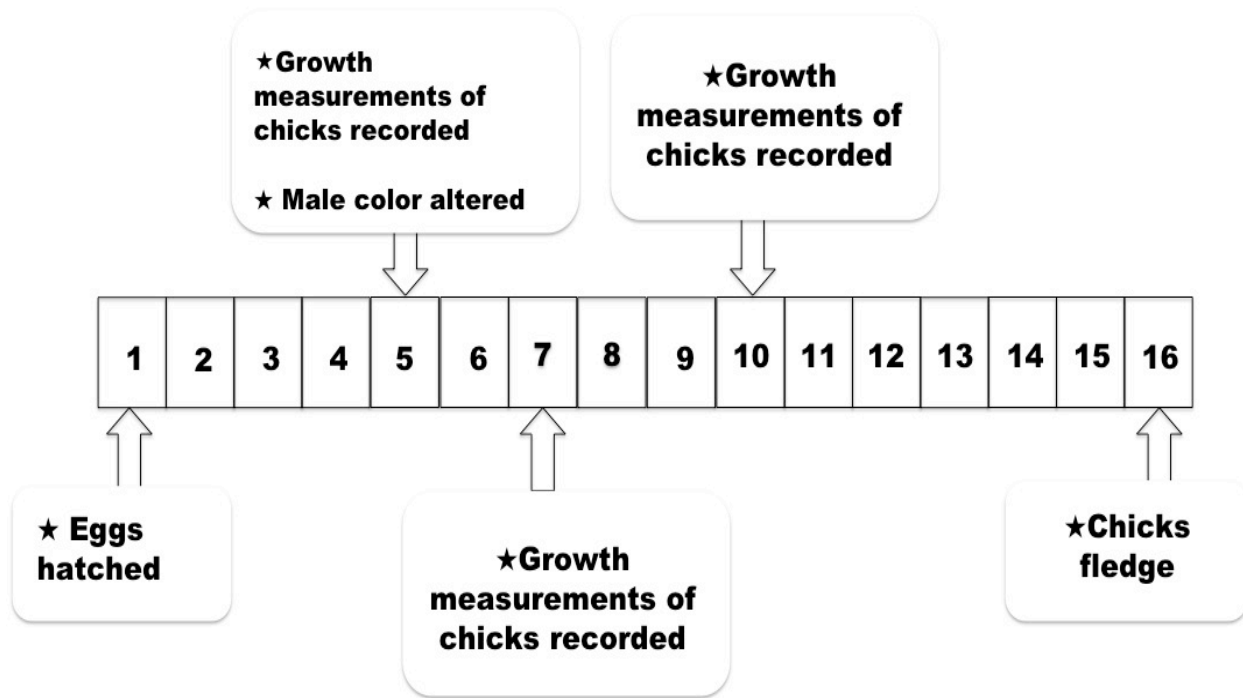
Figure 1. A timeline of Eastern Bluebird nesting sequence and procedures done in the study.

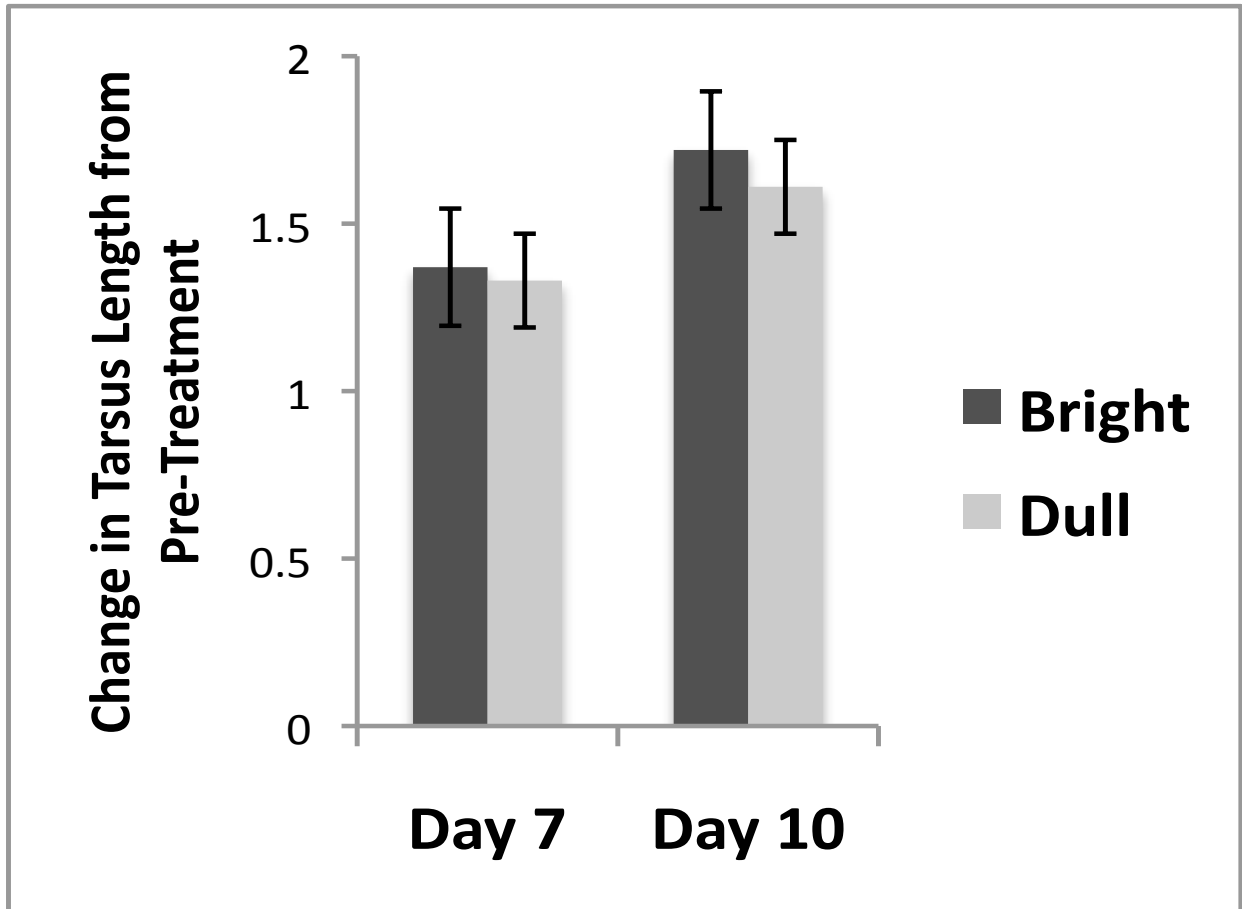Numbers indicate days from the hatching of eggs.

Figure 2. Change in clutch tarsus length of Eastern Bluebird chicks from day 5. The dark

columns represent pairs where the male's plumage brightness was experimentally enhanced. The

light columns represent pairs where the male's plumage brightness was experimentally reduced.

Figure 3. Change in mean body mass of Eastern Bluebird chicks from day 5. The dark columns represent pairs where the male's plumage brightness was experimentally enhanced. The light columns represent pairs where the male's plumage brightness was experimentally reduced.
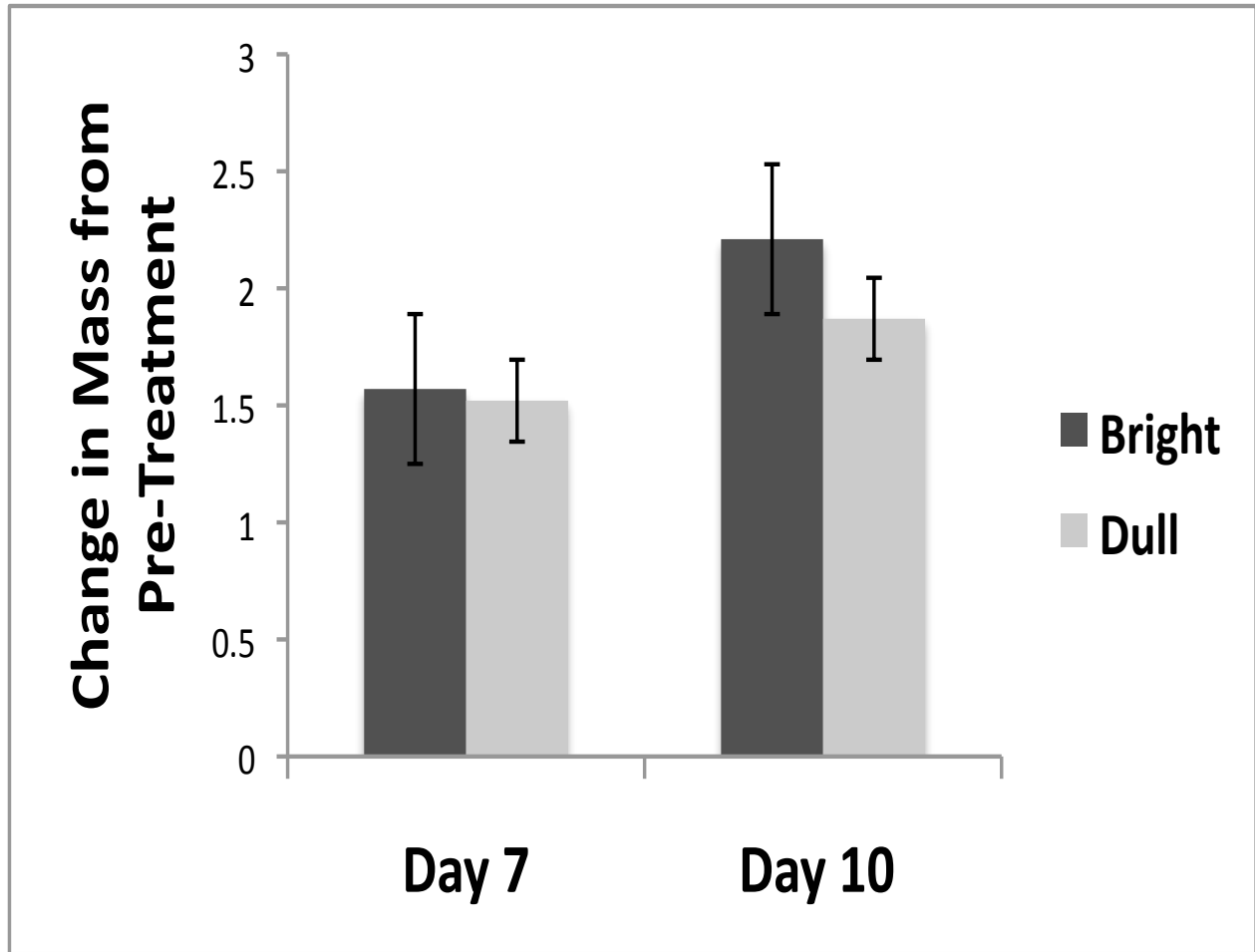
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